IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant(s): Gellman et al. Examiner: Kosar

Title: **HETEROGENEOUS FOLDAMERS CONTAINING α-, β-, AND/OR γ-AMINO ACIDS**

APPELLANT'S APPEAL BRIEF, 37 CFR §41.37

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I certify that this paper is being electronically submitted to the U.S. Patent and Trademark Office via the PAIR (Patent Application Information Retrieval) system on March 13, 2009

Signature:_____ Date: 13 MARCH 2009

REAL PARTY IN INTEREST

The real party in interest is Wisconsin Alumni Research Foundation, which is the owner of the present application by virtue of the assignments recorded at reel 12,952, frame 61, and reel 15,575, frame 72

RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences

STATUS OF CLAIMS

Claims 4, 6, 8, 9, and 11 remain in the application. Claims 8, 9, and 11 currently stand withdrawn from consideration. Claims 1-3, 5, 7, 10, and 12-14 have been canceled.

Claims 4 and 6 are presented for appeal. Claims 4 and 6 stand rejected under 35 USC §101 for allegedly lacking a specific or substantial or well-established utility. Claims 4 and 6 also stand rejected under 35 USC §112, first paragraph, for an alleged lack of enablement because one skilled in the art would not know how to use the claimed compounds.

STATUS OF AMENDMENTS

There was no amendment filed subsequent to the Final Office Action dated August 19, 2008.

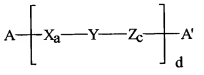
Appellants filed a second Request for Continued Examination, along with a substantive amendment in response to the Final Office Action dated February 1, 2007. That amendment was entered and considered on the merits.

Appellants filed a first Request for Continued Examination, along with a substantive amendment in response to the Final Office Action dated October 28, 2005. That amendment was entered and considered on the merits.

SUMMARY OF CLAIMED SUBJECT MATTER

There are no " means plus function" or "step plus function" elements in the claims.

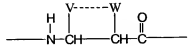
Claims 4 and 6 are on appeal. Both claims 4 and 6 are independent claims that recite isolated, unnatural polypeptide compound of the formula:



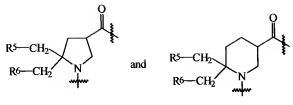
See the specification at page 5, line 28, to page 6, line 9.

The two claims differ in their definition of the X and Z groups. In Claim 4, each X and each Z is independently variable and is selected from the group consisting of α-amino acid residues, β-amino acid residues, and γ-amino acid residues, provided that at least one X or Z is an α-amino acid residue and at least another two of X or Z are two **cyclically-constrained β-amino acid residues**. (Spec., page 10, lines 12-16.) In Claim 6, each X and each Z is independently variable and is selected from the group consisting of α-amino acid residues, β-amino acid residues, and γ-amino acid residues, provided that at least one X or Z is an α-amino acid residue and at least another two of X or Z are two cyclically-constrained residues, the two cyclically-constrained residues being cyclically-constrained β-amino acid residues or cyclically-constrained γ-amino acid residues, or one cyclically-constrained β-amino acid residue and one cyclically-constrained γ-amino acid residue. (Spec. page 5, lines 9-18.)

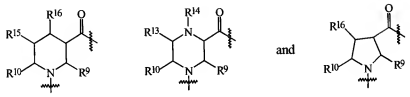
In Claim 4, each cyclically-constrained β-amino acid residue is independently selected from the group consisting of:



(spec, page 10, line 20);



(spec., page 11, line 30); and



(spec., page 13, line 5).

The groups V and W are combined, together with the carbon atoms to which they are bonded, and independently define a substituted or unsubstituted, monocyclic or bicyclic C_3-C_{10} cycloalkyl, cycloalkenyl or heterocyclic ring having one or more N, O or S atom(s) as the heteroatom(s). (Spec., page 10, lines 22-25.) If the ring is substituted, the substituents on carbon atoms of the rings are independently selected from the group consisting of linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, and mono- or bicyclic heteroaryl-C_1-C_6-alkyl. (Spec., page 10, line 26, to page 11, line 2.) The substituents on nitrogen heteroatoms of the rings are independently selected from the group consisting of hydrogen, monocyclic or bicyclic C_1-C_{10}-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, $-S(=O)_2-R^{17}$, $-C(=O)-R^{17}$, $-S(=O)_2-(CH_2)_{n+1}-R^{18}$, and $-C(=O)-(CH_2)_n-R^{18}$, where n = 1 to 6, and wherein R^{17} is independently selected from the group consisting of hydrogen, monocyclic or bicyclic C_1-C_{10}-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic

heteroaryl-C_1-C_6-alkyl. (Spec., page 11, linees 3-9.) R^{18} is independently selected from the group consisting of hydroxy, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl; mono- or bicyclic heteroaryl-C_1-C_6-alkyl; C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane. (Spec., page 126, line 23, to page 127, line 15.)

R^5 and R^6 are independently selected from the group consisting of hydrogen, hydroxy, linear, branched, or cyclic C_1-C_{16}-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_{16} alkylamino; mono- or bicyclic aryl; mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_{16}-alkyl; mono- or bicyclic heteroaryl-C_1-C_{16}-alkyl; $-(CH_2)_{0-6}-OR^7$, $-(CH_2)_{0-6}-SR^7$, $-(CH_2)_{0-6}-S(=O)-CH_2-R^7$, $-(CH_2)_{0-6}-S(=O)_2-CH_2-R^7$, $-(CH_2)_{0-6}-NR^7R^7$, $-(CH_2)_{0-6}-NHC(=O)R^7$, $-(CH_2)_{0-6}-NHS(=O)_2-CH_2-R^7$, $-(CH_2)_{0-6}-C(=O)-OH$, $-(CH_2)_{0-6}-C(=O)-OR^7$, $-(CH_2)_{0-6}-C(=O)-NH_2$, $-(CH_2)_{0-6}-C(=O)-NHR^7$, $-(CH_2)_{0-6}-C(=O)-N(R^7)_2$, $-(CH_2)_{0-6}-O-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-S-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-S(=O)-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-S(=O)_2-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-NH-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-N-\{(CH_2)_{2-6}-R^8\}_2$, $-(CH_2)_{0-6}-NHC(=O)-(CH_2)_{2-6}-R^8$, and $-(CH_2)_{0-6}-NHS(=O)_2-(CH_2)_{2-6}-R^8$. (Spec., page 12, lines 1-10.)

R^7 is independently selected from the group consisting of hydrogen, C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from

N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl. (Spec., page 12, lines 11-14.)

R^8 is selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane. (Spec., page 12, lines 15-29.)

R^9 is selected from the group consisting of linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, -$(CH_2)_{1\text{-}6}$-OR^{11}, -$(CH_2)_{1\text{-}6}$-SR^{11}, -$(CH_2)_{1\text{-}6}$-S(=O)-CH_2-R^{11}, -$(CH_2)_{1\text{-}6}$-S(=O)$_2$-CH_2-R^{11}, -$(CH_2)_{1\text{-}6}$-$NR^{11}R^{11}$, -$(CH_2)_{1\text{-}6}$-NHC(=O)R^{11}, -$(CH_2)_{1\text{-}6}$-NHS(=O)$_2$-CH_2-R^{11}, -$(CH_2)_{0\text{-}6}$-C(=O)-OH, -$(CH_2)_{0\text{-}6}$-C(=O)-OR^{11}, -$(CH_2)_{0\text{-}6}$-C(=O)-NH_2, -$(CH_2)_{0\text{-}6}$-C(=O)-NHR^{11}, -$(CH_2)_{0\text{-}6}$-C(=O)-N(R^{11})$_2$, -$(CH_2)_{1\text{-}6}$-O-$(CH_2)_{2\text{-}6}$-R^{12}, -$(CH_2)_{1\text{-}6}$-S-$(CH_2)_{2\text{-}6}$-R^{12}, -$(CH_2)_{1\text{-}6}$-S(=O)-$(CH_2)_{2\text{-}6}$-R^{12}, -$(CH_2)_{1\text{-}6}$-S(=O)$_2$-$(CH_2)_{2\text{-}6}$-R^{12}, -$(CH_2)_{1\text{-}6}$-NH-$(CH_2)_{2\text{-}6}$-R^{12}, -$(CH_2)_{1\text{-}6}$-N-{$(CH_2)_{2\text{-}6}$-R^{12}}$_2$, -$(CH_2)_{1\text{-}6}$-NHC(=O)-$(CH_2)_{2\text{-}6}$-R^{12}, and -$(CH_2)_{1\text{-}6}$-NHS(=O)$_2$-$(CH_2)_{2\text{-}6}$-R^{12}. (Spec., page 13, lines 10-19.)

R^{10} and R^{13} are independently selected from the group consisting of hydrogen, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl,

mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, $-(CH_2)_{1-6}-OR^{11}$, $-(CH_2)_{1-6}-SR^{11}$, $-(CH_2)_{1-6}-S(=O)-CH_2-R^{11}$, $-(CH_2)_{1-6}-S(=O)_2-CH_2-R^{11}$, $-(CH_2)_{1-6}-NR^{11}R^{11}$, $-(CH_2)_{1-6}-NHC(=O)R^{11}$, $-(CH_2)_{1-6}-NHS(=O)_2-CH_2-R^{11}$, $-(CH_2)_{0-6}-C(=O)-OH$, $-(CH_2)_{0-6}-C(=O)-OR^{11}$, $-(CH_2)_{0-6}-C(=O)-NH_2$, $-(CH_2)_{0-6}-C(=O)-NHR^{11}$, $-(CH_2)_{0-6}-C(=O)-N(R^{11})_2$, $-(CH_2)_{1-6}-O-(CH_2)_{2-6}-R^{12}$, $-(CH_2)_{1-6}-S-(CH_2)_{2-6}-R^{12}$, $-(CH_2)_{1-6}-S(=O)-(CH_2)_{2-6}-R^{12}$, $-(CH_2)_{1-6}-S(=O)_2-(CH_2)_{2-6}-R^{12}$, $-(CH_2)_{1-6}-NH-(CH_2)_{2-6}-R^{12}$, $-(CH_2)_{1-6}-N-\{(CH_2)_{2-6}-R^{12}\}_2$, $-(CH_2)_{1-6}-NHC(=O)-(CH_2)_{2-6}-R^{12}$, and $-(CH_2)_{1-6}-NHS(=O)_2-(CH_2)_{2-6}-R^{12}$. (Spec., page 13, lines 10-19.)

R^{11} is independently selected from the group consisting of hydrogen, C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl. (Spec., page 13, lines 20-24.)

R^{12} is selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane. (Spec., page 13, line 24, to page 14, line 8.)

R^{14} is selected from the group consisting of hydrogen, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or

bicyclic heteroaryl-C_1-C_6-alkyl, -$S(=O)_2$-$(CH_2)_{1-6}$-R^{11}, -$C(=O)R^{11}$, -$S(=O)_2$-$(CH_2)_{2-6}R^{12}$, and -$C(=O)$-$(CH_2)_{1-6}$-R^{12}; wherein R^{11} and R^{12} are as defined above. (Spec., page 14, lines 9-14.)

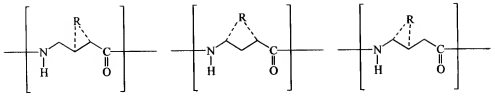
R^{15} and R^{16} are selected from the group listed above for R^{10} and R^{13}, and are further selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane. (Spec., page 14, lines 15-30.)

Each "Y" is a single bond. (Spec., page 15, line 1.)

"A" is independently selected from the group consisting of hydrogen and an amino-terminus protecting group, and "A' " is selected from the group consisting of hydroxy and a carboxy-terminus protecting group. (Spec., page 15, lines 3-5.)

Each "a," "c," and "d" is an independently variable positive integer, and wherein "a" + "c" > 3 (spec., page 15, lines 6-7) or salts thereof (spec., page 15, line 8).

Claim 6 is identical to Claim 4, with the exception of two items. The first item is the definition noted above with respect to the X and Z substituents. The second is that Claim 6 also explicitly specifies the identity of the cyclically-constrained γ-amino acid residues that are possibilities for X and Z. Claim 6 includes the additional requirement that the cyclically-constrained γ-amino acid residues are selected from the group consisting of:



wherein R, together with the carbons to which it is attached, and further together with the β-position carbon in the γ-amino acid backbone where appropriate, independently defines a substituted or unsubstituted, monocyclic or bicyclic C_3 to C_{10} cycloalkyl, cycloalkenyl, or heterocycle moiety, the heterocycle moiety having 1, 2, or 3 heteroatoms selected from the group consisting of N, S, and O. (Spec., page 6, lines 15-22.)

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

I. Whether the companion rejections made under 35 USC §101 (lack of credible utility) and 35 USC §112 (enablement) are improper because the specification articulates a substantial and credible utility for the claimed compounds.

II. Whether the companion rejections made under 35 USC §101 (lack of credible utility) and 35 USC §112 (enablement) are improper because there is a well-known utility for the claimed compounds.

III. Whether the companion rejections made under 35 USC §101 (lack of credible utility) and 35 USC §112 (enablement) are improper because the references relied upon by the Office (the Schmitt reference and the Kim reference, see below) are not contemporaneous with the filing date of the present application, do not describe analogous compounds, and are speculative because the authors did not test the compounds described therein. Therefore the Office's evidence is not probative on the question of utility.

IV. Whether the companion rejections made under 35 USC §101 (lack of credible utility) and 35 USC §112 (enablement) are improper because the Office has improperly discounted the

evidence submitted by the Applicants (including contemporaneous third-party papers and a Rule 132 Declaration of co-inventor Samuel H. Gellman).

ARGUMENT

Introduction:

Practically speaking, there is only one rejection being appealed: the rejection of Claims 4 and 6 under 35 USC §101 on the basis that the claimed compounds lack either a specific and substantial utility or a well-established utility. Claims 4 and 6 also stand rejected under 35 USC §112, first paragraph (enablement). However, the §112 rejection is predicated entirely upon the rejection under §101. See the Office Action dated May 4, 2005, at page 8, penultimate paragraph:

> Since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility... one skilled in the art clearly would not know how to use the claimed invention.

Applicants therefore will focus their argument entirely upon the §101 rejection. Applicants submit that because the rejection under §101 is improper and should be reversed, the rejection under §112, first paragraph (enablement) must also be reversed.

I. The companion rejections under 35 USC §101 (lack of credible utility) and 35 USC §112 (enablement) are improper because the specification articulates a substantial and credible utility for the claimed compounds.

Applicants first raised this ground of traversal starting at page 21 of their response filed August 4, 2005.

The compounds recited in the present claims are unnatural, and contain at least two cyclically-constrained β- and/or γ-amino acid residues. The compounds will **not** adopt the same conformations as naturally-occurring α-polypeptides because they are constrained in a fashion not found in naturally-occurring polypeptides. Nor are the conformations adopted by the claimed compounds exhibited by all compounds or all proteins and/or polypeptides in general. The presently claimed compounds will adopt a more limited number of conformations than analogous α-polypeptides. This is due to the rotational constraints imposed by placing the backbone carbon atoms into a cyclic moiety. But because the

present compounds are composed of peptide bonds, the present compounds do adopt conformations that mimic those of natural polypeptides and proteins. Thus, the claimed compounds have a specific, substantial, and credible utility as peptide mimetics that are not degraded by proteolytic enzymes. This utility is explicitly disclosed in the specification, see the passage spanning page 19, line 19, to page 20, line 25:

> The subject compounds find use as peptide mimetics that are not easily degraded by the action of proteolytic enzymes. Thus, the cyclically-constrained peptides of the present invention can be used as probes to explore protein-protein interactions. Because the compounds of the present invention are cyclically-constrained, they are more restricted conformationally than their strictly α-polypeptide counterparts. The compounds can be labeled and tracked throughout any given reaction. The effect the compound has on any given reaction provides valuable information on either or both of the kinetics and/or thermodynamics of the reaction being studied. Such reactions can be performed *in vitro*, *in vivo*, and *ex vivo*.
>
> Libraries of the subject compounds can also be prepared by automated means, thus providing access to a huge database which can be used as a tool to test, for example, potentially biologically-active agents.
>
> One highly useful aspect of the invention is that because the backbone is heterogenous, a portion of the residues, such as the α-amino acids, provide functional diversity (thus allowing many different types of reactions in many different types of environments to be explored), while the cyclically-constrained residues provide conformational specificity and stability. For example, massive diversity can be obtained using commercially-available α-amino acids as building blocks, while structural rigidity is conferred by using only a single type of rigidified (*i.e.*, cyclically-constrained) β- or γ-amino acid.
>
> With particular focus on protein-protein interactions, it has long been a goal of biological scientists to disrupt specific protein-protein interactions as a means to explore the nature of the interaction. This goal has proven difficult to achieve using traditional small molecules. Binding size is likely part of the problem. Protein-protein complexes generally involve relatively large molecular surfaces. This makes it difficult for a small molecule to bind competitively at such a site. The present compounds, however, are polyamides and can be quite large. Thus, as a class, these compounds, individually and in the form of large libraries of compounds, are much better suited for probing protein-protein interactions than are small molecules. Additionally, the conformations of the subject compounds are periodic; the conformations can be extended simply by adding additional monomers to the polypeptide. Thus, the present compounds can be fabricated as relatively small skeletons or as very large skeletons, the size being dictated, at least in part, by the size of the binding site to be studied.

See also the passage bridging pages 24-25 of the specification:

> The utility of these compounds for probing protein interactions is great because, as noted above, the γ-peptides adopt structures analogous to those seen in natural proteins and peptides. Thus, the subject compounds mimic natural protein conformations in solution, but are resistant or immune to proteolytic degradation by proteases and peptidases. The cyclically-constrained γ-amino acid residues incorporated into homogeneous γ-peptide backbones are useful probes in the study of chemical and enzymatic interactions involving natural proteins. Also, the compounds disclosed herein add greatly to the γ-peptide field, in terms of both the number of alternative secondary structures that can be accessed and the intrinsic stability of those secondary structures. The subject compounds are useful probes because the cyclically-constrained residues create secondary structures with high conformational stability at short oligomer lengths that are also resistant to enzymatic degradation. The invention thus enhances the control over γ-peptide folding preferences, thereby providing a larger "toolbox" of probes to be used in investigating the function of naturally-occurring proteins.

The ability to mimic a naturally-occurring phenomenon, **under tightly controlled conditions**, is specific, substantial, credible, practical and useful. The stated utility is also directed specifically to the present compounds because they are non-natural and structurally rigid. That is, because of the cyclical constraints in the backbone, while the present compounds mimic natural proteins, they also adopt a far smaller set of conformations. The utility of the compounds is directly linked to their structure. Thus, protein-protein binding experiments can be conducted using the claimed compounds under more rigorous conditions than if more labile α-polypeptidic compounds were used.

The *Brenner v. Manson* case, 383 US 519, directly addresses the utility issue. It stands for the proposition that an invention lacks patentable utility if it includes only a general assertion of similarities to known compounds that are known to be useful **without sufficient corresponding explanation why the claimed compounds are believed to be similary useful**. In the present application, however, Applicants clearly explain *why* the compounds are useful (from p. 25 of the specification):

> The subject compounds are useful probes because the cyclically- constrained residues create secondary structures with high conformational stability at short oligomer lengths that are also resistant to enzymatic degradation. The invention thus enhances the

control over γ-peptide folding preferences, thereby providing a larger "toolbox" of probes to be used in investigating the function of naturally-occurring proteins.

Applicants thus submit that the application as filed sets forth a specific, substantial, and credible utility for the claimed compounds. The rejections under §101 and §112, first paragraph are therefore improper and should be reversed.

II. The companion rejections made under 35 USC §101 (lack of credible utility) and 35 USC §112 (enablement) are improper because there is a well-known utility for the claimed compounds.

Applicants first raised this ground of traversal starting at page 24 of their response filed August 4, 2005. See also the passage starting at page 18 of Applicants' response filed January 30, 2006, and the passage starting at page 18 of Applicants' response filed November 3, 2006.

This rejection is improper and should be reversed because the relevant art establishes a specific, substantial, and credible utility for compounds related to those now claimed. According to MPEP §2107.01(II), if there is a well-established utility for the claimed invention, Applicants are entitled to provide evidence of that well-established utility and to rely upon it. As evidence of the utility of the claimed compounds, Applicants submitted for consideration Seebach et al. (2003) "Design and Synthesis of γ-Dipeptide Derivatives with Submicromolar Affinities for Human Somatostatin Receptors," *Angew. Chem. Int. Ed.*, 42(7):776-778. (See reference 1 of the Evidence Appendix, see also Exhibit A to Dr. Gellman's Rule 132 Declaration, reference 6 of the Evidence Appendix.)

Applicants submit that the Office discounted several irrefutable facts regarding the Seebach et al. paper:

(1) The Seebach et al. paper is contemporaneous with the filing date of the present application. The paper was submitted to the publisher on September 25, 2002, prior the actual filing date of the present application, but after Applicants' earliest claimed priority date of August 26, 2002.

(2) The authors of the Seebach et al. paper **did not** rationally design the dipeptide they tested. They simply fabricated it from readily available, commercial starting materials. See page 776 the Seebach et al. paper. The discussion at the left-hand column of 777 of Seebach et al. and continuing to

the top of the right-hand column, however, indicates that Seebach et al. also found evidence that the compounds they tested adopt distinct and stable secondary structures.

(3) Seebach et al. describe their results as "confirmative, surprising, and promising." See page 777, right-hand column, second full paragraph.

In short, Seebach et al's work evidences a real-world, specific, substantial, practical, credible, and significant utility, **in exactly the same fashion** as the subject invention. Applicants therefore submit that the claimed compounds, which share key structural similarities to Seebach's compounds, have a well-known utility in the prior art. Therefore the rejection of Claims 4 and 6 under §101 is improper and should be reversed.

In discounting Applicants' reliance on Seebach et al. to prove a well-established utility for the claimed compounds, the Office replied, in full, as follows:

> Seebach... does not provide utility for Applicants' invention at the time of Applicants' filing. Further, the utility of Seebach is of a specific nature - their ability to mimic binding interactions of two proteins - γ-dipeptides of Seebach and their interaction with human somatostatin receptors, unlike the general utility disclosed [in the subject application] - useful probes for investigating the function of naturally-occurring proteins

See the Office Action dated October 28, 2005, in the paragraph spanning pages 2 and 3, and the Office Action dated October 29, 2007, at page 6, last paragraph..

Applicants submit that the above reasoning is reversible error because the Seebach et al. reference evidences **the exact same utility articulated in the present application**. If a well-established utility exists, Applicants are not required to discuss that utility in their application as filed. See MPEP §2107.01(II). In the above-quoted passage, the Office simply concluded that the Seebach et al. paper does not prove a well-established utility for the claimed compounds, but never directly stated why that is the case. Applicants specifically asked the Office to clarify its position (at page 19, fourth full paragraph of their response filed January 30, 2006). In the Office Action dated May 5, 2006, 4th full paragraph, the Office replied by stating the following:

> [T]he compounds of Seebach are γ-dipeptides, while the instantly claimed compounds are, at a minimum, tetrapeptides with at least 1 α-amino acid and at least two cyclically-constrained β-amino acids. The compounds are not co-extensive or commensurate in

scope, and thus cannot provide a "well-established utility" for the instant compounds based upon structure and amino acid content.

Applicants submit that this statement is reversible error because it applies an impossible-to-satisfy standard for showing a well-established utility. If Applicants were able to produce a contemporaneous document that disclosed compounds that were "co-extensive" in scope with the claimed compounds in order to establish a well-established utility pursuant to MPEP §2107(II), the Office would promptly issue a §102 rejection in view of such a document. In effect, the Office is requiring Applicants to produce an anticipatory reference in order to show that a well-established utility exists for the presently claimed compounds.

In the passage from the bottom of page 6 of the October 29, 2007 Office Action, the Office further stated that Seebach et al. did not prove a utility for using the stated compounds as peptidomimetic drugs. That point, however, is irrelevant because Applicants have not asserted that utility. (Applicants are entitled to assert alternative utilities, and only one utility is required to satisfy §101.)

Applicants submit that the above-noted grounds of rejection are reversible error because the standard articulated by the MPEP is not so stringent with respect to proving a well-established utility. MPEP §2107(II) dictates that an invention has a well-established utility if: (i) a person of ordinary skill in the art would immediately appreciate *why* the invention is useful based on the characteristics of the invention (*e.g.*, properties or applications of a product or process), and (ii) the utility is specific, substantial, and credible. See the discussion starting at page 18, last paragraph, of Applicants' response dated November 3, 2006.

Addressing these two prongs in order: (1) The Seebach et al. reference refers to compounds that are *similar* to those currently claimed. Seebach's compounds are in the same class as the presently claimed compounds, namely polypeptides that contain 1 or 2 extra methylene carbon atoms in the backbone of certain residues, that is, polypeptides containing β-amino acids and/or γ-amino residues. One skilled in the art would immediately recognize that these compounds are unnatural peptido-mimetics in the same fashion as the claimed compounds. See the specification of the present application itself, as well as the Seebach et al. paper. See also the discussion regarding Dr. Gellman's

Rule 132 Declaration, below. (2) The Office has admitted **on the record** that the utility articulated in Seebach et al. is "specific," (and presumably substantial, and credible): "The utility of Seebach is of a specific nature - their ability to mimic binding interactions of two proteins - [the] γ-dipeptide of Seebach and their interaction with human somatostatin receptors." See the bottom of page 2 of the Office Action dated October 28. Seebach's utility **is the same as that articulated in the present application**. Therefore the utility of the claimed compounds is also specific, substantial, and credible.

There are also other contemporaneous documents that show the same well-established utility as articulated in the present application for compounds that are within the same structural class as those now claimed. See, for example, U.S. Patent No. 6,958,384 (Evidence Appendix, reference 6, and Applicant's response dated November 3, 2006 starting at page 19, last paragraph). The '384 Patent is far more relevant on the question of utility than is the Schmitt et al. paper cited by the Office (discussed in the following section) because the Office has admitted on the record that the Schmitt et al. paper is not contemporaneous with the filing of the present application. (See the bottom of page 4 of the Office Action dated May 5, 2006.)

The application that matured into the '384 Patent was published on November 13, 2003 (less than three months after the filing date of the present application). The '384 Patent is therefore contemporaneous in time with the present application. Because it is an issued patent, the '384 Patent and the claims it contains are presumed to be valid and to comply with all of the requirements of §101 and §112. See 35 USC §282: "A patent shall be presumed valid." The Office completely ignored the probative evidentiary value of the '384 Patent, stating only "examiners should not express any opinion on the operability of a patent." See the page 3 Office Action dated October 29, 2007. Applicants, however, were not asking the Office to opine on the operability of the invention claimed in the '384 Patent. Applicants submitted that '384 Patent as proof of a well-established utility for the currently claimed compounds. Again, the utility in the '384 Patent is exactly the same as the utility articulated in the present application. See page 17, second full paragraph, of Applicants' response dated March 31, 2008.

Applicants therefore submit that the third party evidence they supplied to the Office evidences a well-established utility for compounds of the type recited in the present claims. The claimed compounds share this same well-established utility.

Applicants therefore submit that the rejections of the claims under §101 and §112, first paragraph (enablement) are improper. Reversal of the rejections is respectfully requested.

III. The companion rejections under 35 USC §101 (lack of credible utility) and 35 USC §112 (enablement) are improper because the references relied upon by the Office are not contemporaneous with the filing date of the present application, do not describe analogous compounds, are speculative because the authors did not test the compounds described therein, and discuss different utilities than those disclosed in the present application. Therefore the Office's evidence is not probative on the question of utility.

These related issues were raised by the Applicants at page 18, second full paragraph *et seq.* of their Response filed August 1, 2007, and at page 16, middle paragraph et seq. of their Response filed March 31, 2008.

In support of its rejection under §101, the Office cited to Schmitt et al., reference 7 of the Evidence Appendix.

With regard to the Schmitt et al. paper itself, Applicants' point is three-fold:

(1) The Schmitt et al. paper **is not** contemporaneous with the filing date of the present application.

(2) Schmitt et al. did **no** testing of the compounds described therein.

(3) Schmitt et al. *hypothesized* a general utility for the compounds described therein, but Schmitt's compounds are **not** the same as the claimed compounds.

The overarching point is that the Office is supporting its rejection under §101 by citing to a non-contemporaneous paper, that describes non-analogous compounds, and wherein **none** of the compounds were tested for any utility. Applicants respectfully submit that reliance on the Schmitt et al. paper is therefore reversible error.

With respect to the '384 Patent cited by the Applicants to establish a well-known utility for the claimed compounds, the Office stated, on the record, that "a single patent does not establish that the instantly asserted utility was 'well established' such that 'a person of ordinary skill in the art would immediately appreciate why the invention was useful...' . " See the sentence spanning pages 3 and 4 of the Office Action dated October 29, 2007. Yet the Office itself is relying upon a single, extremely

short, non-contemporaneous paper (Schmitt et al.) to discredit the well established utility articulated in the application as filed. Applicants submit that this is reversible error.

Also, Applicants note they are **not** relying **solely** upon U.S. Patent 6,958,384 to establish utility. In discounting the probative value of Dr. Gellman's Rule 132 Declaration, the Office stated (at page 3 of the Final Office Action dated February 1, 2007):

> However, nowhere in the instant specification can the examiner find any reference to blocking Bcl-x_L/BH3 domain interactions. If such a reference were present, with adequate guidance on performing such an experiment - in the instant specification, the compound would likely have utility, as blocking Bcl-x_L/BH3 domain interactions has a specific, substantial, and credible utility.

Applicants note that the Bcl-x_L/BH3 domain model was chosen by Dr. Gellman because the system **is well characterized and well known** to those skilled in the art. See paragraph 10 of Dr. Gellman's Rule 132 Declaration.

The point here is that the Office has indicated that this utility (blocking of Bcl-x_L/BH3 domain interactions) is "likely" sufficient for purposes of §112, first paragraph, enablement, but then discounts this data because it was not included in the application as filed. But MPEP MPEP §2107.01(II) does not require this data to appear in the specification as filed if the utility is well known. Applicants submitted Dr. Gellman's Rule 132 Declaration, as well as Seebach et al. and U.S. Patent 6,958,384 to to show the existence of a well-established utility. Applicants submit that this objective evidence has been improperly downplayed by the Office and therefore the rejection under §101 is untenable.

In support of its §101 rejection, the Office also cited to Kim et al., reference no. 8 of the Evidence Appendix. While the Kim et al. paper is contemporaneous to the present application, the paper uses **different** compounds from those claimed for a **different** utility. The utility Kim et al. were trying to establish was to create a ligand for specific for profilin. This utility is different than what would be the analogous utility in the present application, namely, to disrupt the interaction of profilin with its α-polypeptide binding partner. Therefore the Office's reliance on Kim et al. is misplaced because the Kim et al. paper has no probative evidentiary value with respect to the well-established utility asserted by Applicants.

Lastly, at page 5, second full paragraph of the Office Action dated May 5, 2006, the Office states that while chemical libraries are commercially available, they are sold as "research tools," which are "clearly delineated" by MPEP §2107.01(I) as being a utility which is not substantial. Applicants

submit that this grounds of rejection is reversible error because that there is no such "clear delineation" within the MPEP regarding "research tools." MPEP §2107.01(I)(C) is titled "Research Tools," but this section of the MPEP explicitly states that "Labels such as 'research tool'... **are not helpful** in determining if an applicant has identified a specific and substantial utility for the invention." (Emphasis added.) While perhaps the phrase "research tool" was used only as a shorthand expression, it appears that the Office has labeled the claimed invention a "research tool," and has rejected the claims, at least in part, on that basis. Based on the explicit language of MPEP §2107.01(I)(C), Applicants submit that this is an improper basis upon which to support a rejection under §101.

MPEP §2107.01 indicates that "practical utility" is a shorthand way of attributing "real-world" value to claimed subject matter. In other words, one skilled in the art can use a claimed discovery in a manner which provides some immediate benefit to the public. This section of the MPEP goes on to state that practical considerations require the Office to rely on the inventor's understanding of his or her invention in determining whether and in what regard an invention is believed to be "useful." The Examiners are thus explicitly encouraged by the MPEP to "focus on and be receptive to assertions made by the applicant that an invention is 'useful' for a particular reason."

Applicants therefore submit that the rejections under §101 (utility) and §112, first paragraph (enablement) are improper and should be reversed.

IV. The companion rejections made under 35 USC §101 (lack of credible utility) and 35 USC §112 (enablement) are improper because the Office has discounted the evidence submitted by the Applicants.

In support of it's traversal of the rejection under §101, Applicants provided a Rule 132 Declaration of co-inventor Samuel H. Gellman, which is attached as reference 5 of the Evidence Appendix. Applicants submit that the evidence contained in the declaration further evidences a well-established utility for the claimed compounds and that the evidence was improperly discounted by the Office. The explicit purpose of Dr. Gellman's declaration was: (1) to show that utility described in the application is well-understood by a chemist of ordinary skill; and (2) that the utility is credible and specific to the claimed compounds. Applicants submit that Dr. Gellman's declaration clearly established those two points, and thus the rejection under §101 should be reversed.

Dr. Gellman makes a number of points in his declaration that are directly salient to the question of utility. For example, at paragraph 5 of his declaration, Dr. Gellman notes that the utility of the claimed compounds arises from their structure: "The claimed compounds adopt stable secondary conformation[s]." Dr. Gellman goes on to state that all of the claimed compounds "share the feature of having two or more unnatural amino acid residues, either β-amino acid residues or γ-amino acid residues." Because the compounds adopt stable secondary conformations, "they mimic the pharmacological properties of natural peptides," but are less susceptible to enzymatic degradation" as are natural peptides.

Dr. Gellman directly addressed the Seebach et al. paper at paragraphs 6 and 7 of his declaration, stating that the paper is contemporaneous and addressed compounds are "closely related" to the claimed compounds because Seebach's compounds are constructed of γ-amino acid residues. Dr. Gellman goes on to state that the Seebach et al. paper is relevant to the utility of the present compounds because Seebach et al. made the compounds specifically to mimic the binding interactions between two peptides, which is the same utility stated in the present application. Dr. Gellman goes on to conclude paragraph 6 of his declaration by noting that the Seebach et al. paper "clearly shows" that compounds falling with the same class as those now claimed have a utility that is well-known to peptide chemists (utility as peptido-mimetics) and that that utility is structure-based. The use of β-amino acid residues and γ-amino acid residues imbues the compounds with **structure-specific** utility.

Similarly, in paragraph 7 of his declaration, Dr. Gellman indicates that the '384 Patent discloses the same utility as disclosed in the present application. Thus, the presently claimed compounds have a well-established utility that was readily understood by one of skill in the art at the time the present application was filed.

At paragraph 8 of his declaration, Dr. Gellman further notes that chemical compound libraries are commercially available products. See Exhibits B, C, and D, attached to Dr. Gellman's declaration for evidence of the commercial availability of such chemical libraries. In light of the exhibits, Dr. Gellman concludes that there is a well-established utility for chemical libraries.

With respect to the second prong of MPEP §2107(II), that the utility is specific, substantial, and credible, and bolster not just by the statements in Dr. Gellman's declaration, but clearly evidenced by third party documents, such as the '384 Patent and the Seebach et al. paper. Applicants note that the '384 Patent discloses the same specific, substantial, and credible utility as recited in the present

application. Because the utility described in the '384 Patent is clearly well-established (the patent is available to the public), and because the '384 Patent is presumed to be in compliance with the utility requirement of §101, Applicants submit that the presently claimed compounds likewise have a specific, substantial, and credible utility.

In the Office Action dated May 5, 2006, at page 5, top, the Office took the position that interrupting protein-protein interactions is a general utility, but did not articulate how or why it arrived at that conclusion. Applicants submit this is reversible error. The Office has not provided any basis upon which it concludes that interrupting protein-protein interactions is a "generic" utility.

Applicants also submit that the Office is implicitly requiring Applicants to disclose a utility within the application itself. **There is no such requirement**, and the Office's insistence on this point is reversible error. Where a utility is well-known in the art, there is no requirement that the Applicant actually disclose a utility within the application itself. When a §101 rejection is made by the Office, the burden is shifted to the Applicant to establish that a specific and substantial utility was well-established at the time of filing. See MPEP 2107(II)(B)(3)(ii).

At page 5 of the Office Action dated May 5, 2006 (first full paragraph), the Office asked "which specific protein-protein interactions are contemplated and disclosed to be disrupted by Applicant?" And "To what end are the interactions disrupted?" Dr. Gellman's declaration was submitted to answer these two questions directly, but the evidence was improperly dismissed by the Office. At paragraphs 9 through 19 of his declaration, Dr. Gellman described a series of experiments showing that compounds according to the present invention can block Bcl-x_L/BH3 domain interactions. As noted at the end of paragraph 10 of his declaration, the results of the experiments show that foldamer-based designs can provide tight-binding ligands for a large protein-recognition site (K_i for compound 4 = 0.7 nM). The tight binding of chimeric ($\alpha/\beta+\alpha$)-peptides (peptides within the scope of the present claims) to Bcl-x_L suggests that combining different foldamer scaffolds is also an effective (and perhaps general) strategy for protein ligand design.

The conclusions at paragraph 19 of his declaration are particularly relevant to the question of specific, substantial, and credible utility. Notably, Dr. Gellman concludes that the results of the experiments are significant because they reflect the exact utility that is stated in the application. The subject compounds mimic natural protein conformations in solution, but are resistant or immune to

proteolytic degradation by proteases and peptidases. The compounds are thus useful in the study of chemical and enzymatic interactions involving natural proteins.

At paragraph 20 of his declaration, Dr. Gellman also includes data showing that compounds according to the present invention inhibit viral infection. Here, α/β-peptides falling within the scope of the present claims are shown to inhibit the infection of human fibroblast cells with human cytomegalovirus (CMV), which is a source of human disease. Dr. Gellman includes data for several compounds in a graph (Figure 6 of the declaration). (The structures of the compounds tested are shown in Figure 7.) As Dr. Gellman states at paragraph 20, in this assay, the virus expresses green fluorescent protein (GFP). The green fluorescence is thus tracked to learn how many cells have been infected with the virus. For compounds VI-139 and VI-145, the data show significant inhibition of viral infection when the α/β-peptide is present at 50 μM. This results are significant because, as Dr. Gellman states, the results show that α/β-peptides according to the present claims can block CMV infection of target cells, which is a biomedically valuable, structure-specific property.

In terms of substantiality, Dr. Gellman's declaration clearly shows that compounds falling within the scope of the present claims can disrupt protein-protein interactions in Bcl-2 proteins, an anti-apoptotic protein that plays a critical role in the proliferation of many cancers. Compounds falling within the scope of the present claims can also inhibit the infection of fibroblast cells by cytomegalovirus. These are substantial, real-world, specific utilities. As noted by the MPEP §2101.01 itself, **any** reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient, at least with regard to defining a "substantial" utility. Applicants respectfully submit that the evidence submitted herewith corroborates the specific, substantial, and credible utility set forth in the application as filed.

Applicants therefore respectfully submit that the rejection of the claims under §101 and §112, first paragraph (enablement) be reversed.

CONCLUSION

In light of the reversible errors noted above, Applicants request that the rejection of the claims under §101 and §112, first paragraph (enablement) be reversed and the application passed on to issuance.

Respectfully submitted,

13 MARCH 2009

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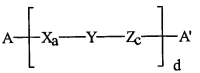
CLAIMS APPENDIX

1-3. (CANCELED)

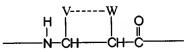
4. (PREVIOUSLY PRESENTED) An isolated, unnatural polypeptide compound of formula:

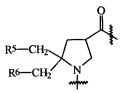


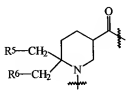
wherein:

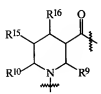
each X and each Z is independently variable and is selected from the group consisting of α-amino acid residues, β-amino acid residues, and γ-amino acid residues, provided that at least one X or Z is an α-amino acid residue and at least another two of X or Z are two cyclically-constrained β-amino acid residues; and

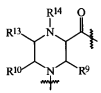
wherein each cyclically-constrained β-amino acid residue is independently selected from the group consisting of:











and

wherein V and W are combined, together with the carbon atoms to which they are bonded, and independently define a substituted or unsubsituted, monocyclic or bicyclic C_3-C_{10} cycloalkyl, cycloalkenyl or heterocyclic ring having one or more N, O or S atom(s) as the heteroatom(s);

the substituents on carbon atoms of the rings being independently selected from the group consisting of linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, and mono- or bicyclic heteroaryl-C_1-C_6-alkyl;

the substituents on nitrogen heteroatoms of the rings being independently selected from the group consisting of hydrogen, monocyclic or bicyclic C_1-C_{10}-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, $-S(=O)_2-R^{17}$, $-C(=O)-R^{17}$, $-S(=O)_2-(CH_2)_{n+1}-R^{18}$, and $-C(=O)-(CH_2)_n-R^{18}$, where n = 1 to 6;

wherein R^{17} is independently selected from the group consisting of hydrogen, monocyclic or bicyclic C_1-C_{10}-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl; and

wherein R^{18} is independently selected from the group consisting of hydroxy, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl; mono- or bicyclic heteroaryl-C_1-C_6-alkyl; C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or

diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane; and

wherein R^5 and R^6 are independently selected from the group consisting of hydrogen, hydroxy, linear, branched, or cyclic C_1-C_{16}-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_{16} alkylamino; mono- or bicyclic aryl; mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_{16}-alkyl; mono- or bicyclic heteroaryl-C_1-C_{16}-alkyl; -$(CH_2)_{0-6}$-OR^7, -$(CH_2)_{0-6}$-SR^7, -$(CH_2)_{0-6}$-S(=O)-CH_2-R^7, -$(CH_2)_{0-6}$-$S(=O)_2$-CH_2-R^7, -$(CH_2)_{0-6}$-NR^7R^7, -$(CH_2)_{0-6}$-NHC(=O)R^7, -$(CH_2)_{0-6}$-$NHS(=O)_2$-CH_2-R^7, -$(CH_2)_{0-6}$-C(=O)-OH, -$(CH_2)_{0-6}$-C(=O)-OR^7, -$(CH_2)_{0-6}$-C(=O)-NH_2, -$(CH_2)_{0-6}$-C(=O)-NHR^7, -$(CH_2)_{0-6}$-C(=O)-$N(R^7)_2$, -$(CH_2)_{0-6}$-O-$(CH_2)_{2-6}$-R^8, -$(CH_2)_{0-6}$-S-$(CH_2)_{2-6}$-R^8, -$(CH_2)_{0-6}$-S(=O)-$(CH_2)_{2-6}$-R^8, -$(CH_2)_{0-6}$-$S(=O)_2$-$(CH_2)_{2-6}$-R^8, -$(CH_2)_{0-6}$-NH-$(CH_2)_{2-6}$-R^8, -$(CH_2)_{0-6}$-N-$\{(CH_2)_{2-6}$-$R^8\}_2$, -$(CH_2)_{0-6}$-NHC(=O)-$(CH_2)_{2-6}$-R^8, and -$(CH_2)_{0-6}$-$NHS(=O)_2$-$(CH_2)_{2-6}$-R^8; wherein

R^7 is independently selected from the group consisting of hydrogen, C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl; and

R^8 is selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-

heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane; and

wherein R^9 is selected from the group consisting of linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, -$(CH_2)_{1-6}$-OR^{11}, -$(CH_2)_{1-6}$-SR^{11}, -$(CH_2)_{1-6}$-S(=O)-CH_2-R^{11}, -$(CH_2)_{1-6}$-S(=O)$_2$-CH_2-R^{11}, -$(CH_2)_{1-6}$-$NR^{11}R^{11}$, -$(CH_2)_{1-6}$-NHC(=O)R^{11}, -$(CH_2)_{1-6}$-NHS(=O)$_2$-CH_2-R^{11}, -$(CH_2)_{0-6}$-C(=O)-OH, -$(CH_2)_{0-6}$-C(=O)-OR^{11}, -$(CH_2)_{0-6}$-C(=O)-NH_2, -$(CH_2)_{0-6}$-C(=O)-NHR^{11}, -$(CH_2)_{0-6}$-C(=O)-N(R^{11})$_2$, -$(CH_2)_{1-6}$-O-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-S-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-S(=O)-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-S(=O)$_2$-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-NH-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-N-{$(CH_2)_{2-6}$-R^{12}}$_2$, -$(CH_2)_{1-6}$-NHC(=O)-$(CH_2)_{2-6}$-R^{12}, and -$(CH_2)_{1-6}$-NHS(=O)$_2$-$(CH_2)_{2-6}$-R^{12};

R^{10} and R^{13} are independently selected from the group consisting of hydrogen, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, -$(CH_2)_{1-6}$-OR^{11}, -$(CH_2)_{1-6}$-SR^{11}, -$(CH_2)_{1-6}$-S(=O)-CH_2-R^{11}, -$(CH_2)_{1-6}$-S(=O)$_2$-CH_2-R^{11}, -$(CH_2)_{1-6}$-$NR^{11}R^{11}$, -$(CH_2)_{1-6}$-NHC(=O)R^{11}, -$(CH_2)_{1-6}$-NHS(=O)$_2$-CH_2-R^{11}, -$(CH_2)_{0-6}$-C(=O)-OH, -$(CH_2)_{0-6}$-C(=O)-OR^{11}, -$(CH_2)_{0-6}$-C(=O)-NH_2, -$(CH_2)_{0-6}$-C(=O)-NHR^{11}, -$(CH_2)_{0-6}$-C(=O)-N(R^{11})$_2$, -$(CH_2)_{1-6}$-O-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-S-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-S(=O)-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-S(=O)$_2$-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-NH-$(CH_2)_{2-6}$-R^{12}, -$(CH_2)_{1-6}$-N-{$(CH_2)_{2-6}$-R^{12}}$_2$, -$(CH_2)_{1-6}$-NHC(=O)-$(CH_2)_{2-6}$-R^{12}, and -$(CH_2)_{1-6}$-NHS(=O)$_2$-$(CH_2)_{2-6}$-R^{12}; wherein

R^{11} is independently selected from the group consisting of hydrogen, C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl; and

R^{12} is selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-

alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane;

R^{14} is selected from the group consisting of hydrogen, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, -$S(=O)_2$-$(CH_2)_{1-6}$-R^{11}, -$C(=O)R^{11}$, -$S(=O)_2$-$(CH_2)_{2-6}R^{12}$, and -$C(=O)$-$(CH_2)_{1-6}$-R^{12}; wherein R^{11} and R^{12} are as defined above;

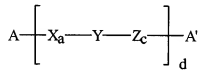
R^{15} and R^{16} are selected from the group listed above for R^{10} and R^{13}, and are further selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane and

"A" is independently selected from the group consisting of hydrogen and an amino-terminus protecting group, and "A' " is selected from the group consisting of hydroxy and a carboxy-terminus protecting group; and

each "a," "c," and "d" is an independently variable positive integer, and wherein "a" + "c" > 3; or salts thereof.

5. (CANCELED)

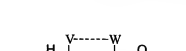
6. (PREVIOUSLY PRESENTED) An isolated, unnatural polypeptide compound of formula:

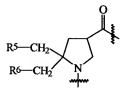


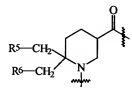
wherein:

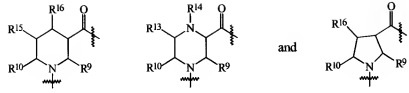
each X and each Z is independently variable and is selected from the group consisting of α-amino acid residues, β-amino acid residues, and γ-amino acid residues, provided that at least one X or Z is an α-amino acid residue and at least another two of X or Z are two cyclically-constrained residues, the two cyclically-constrained residues being cyclically-constrained β-amino acid residues or cyclically-constrained γ-amino acid residues, or one cyclically-constrained β-amino acid residue and one cyclically-constrained γ-amino acid residue; and

wherein the cyclically-constrained β-amino acid residues are selected from the group consisting of:









wherein V and W are combined, together with the carbon atoms to which they are bonded, and independently define a substituted or unsubsituted, monocyclic or bicyclic C_3-C_{10} cycloalkyl, cycloalkenyl or heterocyclic ring having one or more N, O or S atom(s) as the heteroatom(s);

the substituents on carbon atoms of the rings being independently selected from the group consisting of linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, and mono- or bicyclic heteroaryl-C_1-C_6-alkyl

the substituents on nitrogen heteroatoms of the rings being independently selected from the group consisting of hydrogen, monocyclic or bicyclic C_1-C_{10}-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, $-S(=O)_2-R^{17}$, $-C(=O)-R^{17}$, $-S(=O)_2-(CH_2)_{n+1}-R^{18}$, and $-C(=O)-(CH_2)_n-R^{18}$, where n = 1 to 6;

wherein R^{17} is independently selected from the group consisting of hydrogen, monocyclic or bicyclic C_1-C_{10}-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl; and

wherein R^{18} is independently selected from the group consisting of hydroxy, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl; mono- or bicyclic heteroaryl-C_1-C_6-alkyl; C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-

alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane; and

wherein R^5 and R^6 are independently selected from the group consisting of hydrogen, hydroxy, linear, branched, or cyclic C_1-C_{16}-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_{16} alkylamino; mono- or bicyclic aryl; mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_{16}-alkyl; mono- or bicyclic heteroaryl-C_1-C_{16}-alkyl; $-(CH_2)_{0-6}-OR^7$, $-(CH_2)_{0-6}-SR^7$, $-(CH_2)_{0-6}-S(=O)-CH_2-R^7$, $-(CH_2)_{0-6}-S(=O)_2-CH_2-R^7$, $-(CH_2)_{0-6}-NR^7R^7$, $-(CH_2)_{0-6}-NHC(=O)R^7$, $-(CH_2)_{0-6}-NHS(=O)_2-CH_2-R^7$, $-(CH_2)_{0-6}-C(=O)-OH$, $-(CH_2)_{0-6}-C(=O)-OR^7$, $-(CH_2)_{0-6}-C(=O)-NH_2$, $-(CH_2)_{0-6}-C(=O)-NHR^7$, $-(CH_2)_{0-6}-C(=O)-N(R^7)_2$, $-(CH_2)_{0-6}-O-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-S-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-S(=O)-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-S(=O)_2-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-NH-(CH_2)_{2-6}-R^8$, $-(CH_2)_{0-6}-N-\{(CH_2)_{2-6}-R^8\}_2$, $-(CH_2)_{0-6}-NHC(=O)-(CH_2)_{2-6}-R^8$, and $-(CH_2)_{0-6}-NHS(=O)_2-(CH_2)_{2-6}-R^8$; wherein

R^7 is independently selected from the group consisting of hydrogen, C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl; and

R^8 is selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-

alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane;

wherein R^9 is selected from the group consisting of linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, $-(CH_2)_{1\text{-}6}$-OR^{11}, $-(CH_2)_{1\text{-}6}$-SR^{11}, $-(CH_2)_{1\text{-}6}$-S(=O)-CH_2-R^{11}, $-(CH_2)_{1\text{-}6}$-$S(=O)_2$-CH_2-R^{11}, $-(CH_2)_{1\text{-}6}$-$NR^{11}R^{11}$, $-(CH_2)_{1\text{-}6}$-NHC(=O)R^{11}, $-(CH_2)_{1\text{-}6}$-NHS(=O)$_2$-CH_2-R^{11}, $-(CH_2)_{0\text{-}6}$-C(=O)-OH, $-(CH_2)_{0\text{-}6}$-C(=O)-OR^{11}, $-(CH_2)_{0\text{-}6}$-C(=O)-NH_2, $-(CH_2)_{0\text{-}6}$-C(=O)-NHR^{11}, $-(CH_2)_{0\text{-}6}$-C(=O)-$N(R^{11})_2$, $-(CH_2)_{1\text{-}6}$-O-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-S-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-S(=O)-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-$S(=O)_2$-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-NH-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-N-$\{(CH_2)_{2\text{-}6}$-$R^{12}\}_2$, $-(CH_2)_{1\text{-}6}$-NHC(=O)-$(CH_2)_{2\text{-}6}$-R^{12}, and $-(CH_2)_{1\text{-}6}$-NHS(=O)$_2$-$(CH_2)_{2\text{-}6}$-R^{12}; and

R^{10} and R^{13} are independently selected from the group consisting of hydrogen, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, $-(CH_2)_{1\text{-}6}$-OR^{11}, $-(CH_2)_{1\text{-}6}$-SR^{11}, $-(CH_2)_{1\text{-}6}$-S(=O)-CH_2-R^{11}, $-(CH_2)_{1\text{-}6}$-S(=O)$_2$-CH_2-R^{11}, $-(CH_2)_{1\text{-}6}$-$NR^{11}R^{11}$, $-(CH_2)_{1\text{-}6}$-NHC(=O)R^{11}, $-(CH_2)_{1\text{-}6}$-NHS(=O)$_2$-CH_2-R^{11}, $-(CH_2)_{0\text{-}6}$-C(=O)-OH, $-(CH_2)_{0\text{-}6}$-C(=O)-OR^{11}, $-(CH_2)_{0\text{-}6}$-C(=O)-NH_2, $-(CH_2)_{0\text{-}6}$-C(=O)-NHR^{11}, $-(CH_2)_{0\text{-}6}$-C(=O)-$N(R^{11})_2$, $-(CH_2)_{1\text{-}6}$-O-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-S-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-S(=O)-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-$S(=O)_2$-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-NH-$(CH_2)_{2\text{-}6}$-R^{12}, $-(CH_2)_{1\text{-}6}$-N-$\{(CH_2)_{2\text{-}6}$-$R^{12}\}_2$, $-(CH_2)_{1\text{-}6}$-NHC(=O)-$(CH_2)_{2\text{-}6}$-R^{12}, and $-(CH_2)_{1\text{-}6}$-NHS(=O)$_2$-$(CH_2)_{2\text{-}6}$-R^{12}; wherein

R^{11} is independently selected from the group consisting of hydrogen, C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5

heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl; and

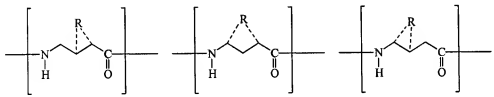
R^{12} is selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane;

R^{14} is selected from the group consisting of hydrogen, linear, branched, or cyclic C_1-C_6-alkyl, alkenyl, or alkynyl; mono-or di- C_1–C_6 alkylamino, mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, $-S(=O)_2-(CH_2)_{1\text{-}6}-R^{11}$, $-C(=O)R^{11}$, $-S(=O)_2-(CH_2)_{2\text{-}6}R^{12}$, and $-C(=O)-(CH_2)_{1\text{-}6}-R^{12}$; wherein R^{11} and R^{12} are as defined above;

R^{15} and R^{16} are selected from the group listed above for R^{10} and R^{13}, and are further selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-

heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane; and

wherein the cyclically-constrained γ-amino acid residues are selected from the group consisting of:



wherein R, together with the carbons to which it is attached, and further together with the β-position carbon in the γ-amino acid backbone where appropriate, independently defines a substituted or unsubstituted, monocyclic or bicyclic C_3 to C_{10} cycloalkyl, cycloalkenyl, or heterocycle moiety, the heterocycle moiety having 1, 2, or 3 heteroatoms selected from the group consisting of N, S, and O; and

each "Y" is a single bond; and

"A" is independently selected from the group consisting of hydrogen and an amino-terminus protecting group, and "A' " is selected from the group consisting of hydroxy and a carboxy-terminus protecting group; and

each "a," "c," and "d" is an independently variable positive integer, and wherein "a" + "c" > 3; or

salts thereof.

7. (CANCELED)

8. (WITHDRAWN, ORIGINAL) The compound of Claim 6, wherein at least one of X or Z is a cyclically-constrained β-amino acid residue wherein V and W, and the carbon atoms to which they are bonded, define a substituted or unsubstituted C_4 to C_6 cycloalkyl, cycloalkenyl, or heterocyclic ring having one nitrogen atom as the sole heteratom.

9. (WITHDRAWN, ORIGINAL) The compound of Claim 6, wherein at least one of X or Z is a cyclically-constrained β-amino acid residue wherein V and W, and the carbon atoms to which they are bonded, define a substituted or unsubstituted cyclopentyl, cyclohexyl, pyrrolidinyl, or piperdinyl ring.

10. (CANCELED)

11. (WITHDRAWN, PREVIOUSLY PRESENTED) A method of probing, disrupting, or mimicking binding interactions between two protein molecules or fragments thereof, the method comprising:

in an *in vivo*, *in vitro*, or *ex vivo* reaction between the two proteins,

(a) introducing to the reaction an unnatural polypeptide compound according to Claim 4; and then

(b) quantifying any effect of the added compound from step (a) on thermodynamic or kinetic parameters of the binding interaction between the two protein molecules or fragments thereof.

12-14. (CANCELED)

EVIDENCE APPENDIX

1. Seebach et al. (2003) "Design and Synthesis of γ-Dipeptide Derivatives with Submicromolar Affinities for Human Somatostatin Receptors," *Angew. Chem. Int. Ed.* 42(7): 776-778.

Included as an attachment to Applicants' response filed August 4, 2005, and subsequently tendered to the Office in a supplemental information disclosure statement filed November 3, 2006.

2. Screen shot from the web page of ChemBridge Corporation (San Diego, California).

Included as Exhibit B to Applicants' response filed January 30, 2006, and subsequently tendered to the Office in a supplemental information disclosure statement filed November 3, 2006.

3. Screen shot from NanoSyn Corporation (Menlo Park, California).

Included as Exhibit C to Applicants' response filed January 30, 2006, and subsequently tendered to the Office in a supplemental information disclosure statement filed November 3, 2006.

4. Screen shot from ActiMol Corporation (Newark, Delaware).

Included as Exhibit D to Applicants' response filed January 30, 2006, and subsequently tendered to the Office in a supplemental information disclosure statement filed November 3, 2006.

5. Rule 132 Declaration of Co-Inventor Samuel H. Gellman.and Exhibits A, B, C, and D. attached thereto.

Included as part of Applicants' response filed November 3, 2006, and acknowledged in the Office Action dated February 1, 2007 at page 3, second full paragraph.

6. U.S. Patent No. 6,958,384.

Included as Exhibit A of Applicants' response filed November 3, 2006.

7. Schmitt et al. (2005), "Unexpected Relationships between Structure and Function in α,β-Peptides: Antimicrobial Foldamers with Heterogenous Backbones," *J. Amer. Chem. Soc.* 127:13130-13131.

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8. Kim et al.(2000), "Synthesis of (3R)-Carboxy Pyrrolidine (a β-Proline Analogue) and its Oligomer," *Bioorg. Med. Chem.*10:2417-2419.

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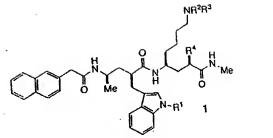
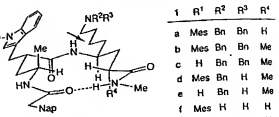
REFERENCE 1

[16] We found that BRP-H is also a polymerization catalyst for other thiophenes, for example, 3-dodecyl-thiophene.
[17] *Handbook of Oligo- and Polythiophenes* (Ed.: D. Fichou), Wiley-VCH, Weinheim, 1999.
[18] D. Dini, E. Decker, F. Andreani, E. Salatelli, P. Hapiot, *Polymer* 2000, *41*, 6473.
[19] In the measurement setup an argon ion laser beam was focused by a 25× microscope objective to a 50 μm diameter spot on the sample. The fluorescence emission was imaged by a 50× objective on a CCD camera.
[20] No UV/Vis spectrum could be recorded from a single vesicle, nor from a suspension of vesicles, because of scattering problems.
[21] A 0.5 g L^{-1} solution of PS-PIAT dissolved in THF was injected into a 30 mg L^{-1} solution of CAL B enzymes; the final water/THF ratio was 12:1 (v/v). After the mixture was left for two days to equilibrate, it was dialyzed to dispose of all noninclueded enzymes.



1	R^1	R^2	R^3	R^4
a	Mes	Bn	Bn	H
b	Mes	Bn	Bn	Me
c	H	Bn	Bn	Me
d	Mes	Bn	H	Me
e	H	Bn	H	Me
f	Mes	H	H	H
g	H	H	H	H



Expected conformation of 1

Scheme 1. Structural formulae of the γ-peptides 1. In the expected conformation of 1 the red arrow points to a CH_3 group of the $H_2N(CH_2)_4$ unit, which is placed inside the shielding cone of the aromatic indole ring. Mes = mesitylenesulfonyl, Bn = benzyl, Nap = naphthyl.

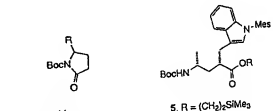
Somatostatin Mimics

Design and Synthesis of γ-Dipeptide Derivatives with Submicromolar Affinities for Human Somatostatin Receptors

Dieter Seebach, Laurent Schaeffer, Meinrad Brenner, and Daniel Hoyer*

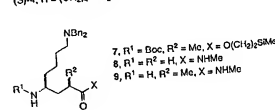
In a previous paper we have shown that simple *N*-acyl-γ-dipeptide amides that resemble a βII′ turn of an α-peptide can be designed to form a turn structure in solution (NMR) and in the solid state (X-ray).[1,2] To see whether such a turn could also be used to mimic a peptide, the biological activity of which rests upon a turn structure carrying functionalized side chains, we have now synthesized compounds **1a–g** (Scheme 1), with the side chain of tryptophan in the Y^2 position of the first and of lysine in the Y^4 position of the second γ-amino acid, and have tested their affinities for the human somatostatin receptors $hsst_{1-5}$.[3–6]

The synthesis of γ-dipeptide derivatives **1** commenced with the *N*-Boc-γ-lactams **2** and **3** (Boc = *tert*-butoxycarbonyl), readily available from the corresponding commercial (*R*)-Ala and (*S*)-Lys acids by known procedures.[1,7] Ring opening (with the Lys derivative after change of side-chain protection, →**4**), and esterification with $Me_3Si(CH_2)_2OH$ provided the (*R*)-Boc-Y^4-hhAla and Boc-Y^4-hhLys(Bn_2) esters, which were



(*R*)-2, R = Me
(*S*)-3, R = $(CH_2)_4NH(2\text{-Cl-Z})$
(*S*)-4, R = $(CH_2)_4NBn_2$

5, R = $(CH_2)_2SiMe_3$
6, R = H



7, R^1 = Boc, R^2 = Me, X = $O(CH_2)_2SiMe_3$
8, R^1 = R^2 = H, X = NHMe
9, R^1 = H, R^2 = Me, X = NHMe

doubly deprotonated and alkylated with 1-mesitylenesulfonyl-3-bromomethylindole and MeI to give the *unlike* $Y^{2,4}$-amino acid derivatives **5** and **7**, respectively. The ester group in compound **5** with Trp side chain was cleaved (Bu_4NF, →**6**), and the lysine-derived esters were converted to the methylamides **8** and **9** without and with 2-methyl substitution, respectively (1. Bu_4NF, 2. $MeNH_2$, 3. F_3CCO_2H). Coupling of the two γ-amino acid derivatives (**6** + **8** and **6** + **9**), removal of the Boc groups, and acylation with 2-naphthylacetic acid[8] (4-methylmorpholine, 1-hydroxy-1*H*-benzotriazole, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) produced the side-chain-protected *N*-acyl-dipeptide amides **1a** and **1b**. Deprotection procedures ($MeSO_3H$, F_3CCO_2H, and Pd/C, H_2) led to the various partially or fully deprotected γ-dipeptide derivatives **1c–1g**. All compounds were purified and fully characterized by elemental analyses, specific optical rotations, circular dichroism (CD), IR, and NMR spectroscopy, and mass spectrometry.

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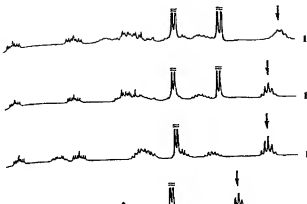

Figure 1. High-field part of the 500 MHz ^{1}H NMR spectra of the γ-dipeptides **1c**, **1e**, **1f**, and **1g** in CD_3OD. The red arrows point to high-field-shifted $N(CH_2)_4$ resonances.

A typical feature of the turn structure in somatostatin and its analogues is the juxtaposition of the tryptophan and lysine side chains, which places CH_2 groups of the $H_2N(CH_2)_4$ unit inside the shielding cone of the aromatic indole ring (NMR shifts between $\delta = 0.8$ and 0.3 ppm are observed).[9] High-field sections of the NMR spectra of four γ-dipeptide amides, shown in Figure 1, in which CH_2 signals appear between $\delta = 0.9$ and 0.6 ppm, confirm the proximity between the corresponding side chains, and are thus compatible with a turn conformation of these compounds. The CD spectra of the *N*-naphthylacetyl dipeptide amides **1** exhibit an intensive negative Cotton effect near 200 nm ([Θ] up to 70000 deg cm^2 dmol^{-1}), with a weaker and broader peak near 220 nm ([Θ] up to 30000 deg cm^2 dmol^{-1}) (Figure 2); this CD pattern may be taken as another piece of evidence for the presence of a secondary structure.

Probably the most stringent test of the γ-dipeptide structure is the affinity for somatostatin receptors. Binding affinities for the five cloned human receptors $hsst_{1-5}$, expressed in CCL-39 cell lines, were determined by displacement of [^{125}I]LTT-SRIF$_{28}$ from these receptor proteins.[10] While the fully protected γ-dipeptide **1d** binds to $hsst_1$ and $hsst_3$ with remarkable K_D values of 0.55 and 1.00 μM, respectively, the partially and the fully deprotected γ-dipeptide derivatives **1f** and **1g** bind to $hsst_2$ with K_D values of 0.51 and 0.87 μM, respectively (Table 1). Intriguingly, the highest affinities (**1d**/$hsst_1$, **1f**/$hsst_2$) are observed when the side chain functional groups (3-indolylmethyl and $(CH_2)_4NH_3^+$) are protected by bulky aromatic moieties (*N*-mesitylenesulfonyl and/or -benzyl)!

The results presented here are confirmative, surprising, and promising; they demonstrate that a 14-amino-acid cyclic disulfide hormone, somatostatin, can be mimicked by a simple, designed, low-molecular-weight, open-chain γ-dipeptide derivative (cf. **1g**) that contains only three amide bonds; they suggest that hitherto unknown hydrophobic pockets are present in the receptors ($hsst_1$, $hsst_3$, and $hsst_4$), which supposedly house the turn-bound Trp and Lys side chains (cf. **1c**, **1d**, **1f**); and they promise a potential of γ-peptides for the development of peptidase-resistant[11] peptidomimetic drugs.

Table 1: pK_D Values for γ-peptides **1b**–**1g** at the five hsst receptors expressed in CCL-39 cells and measured by radioligand binding assays with [^{125}I]LTT-SRIF$_{28}$ as radioligand.[9,10]

Receptor	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	Octreotide[a]	SRIF$_{14}$[a]
$hsst_1$	5.47	6.06	6.26	5.61	5.98	4.73	6.45	9.08
$hsst_2$	<5	<5	5.17	<5	5.01	2.81	9.11	10.06
$hsst_3$	5.53	5.89	6.00	5.73	5.67	5.42	8.60	9.67
$hsst_4$	4.67	5.74	5.92	5.66	5.79	5.44	5.76	8.39
$hsst_5$	4.49	5.01	5.87	5.14	6.29	6.06	7.31	9.01

[a] Submicromolar affinities are highlighted in red. [b] Sandostatin. [c] Somatostatin.

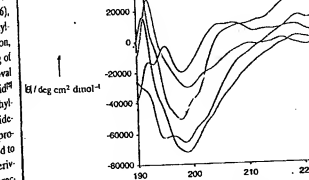

Figure 2. Nonnormalized CD spectra in MeOH (0.2 mM) of the γ-dipeptide derivatives **1b**–**1g**.

Received: September 25, 2002 [Z50242]

[1] M. Brenner, D. Seebach, *Helv. Chim Acta* **2001**, *84*, 2155–2166.
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[5] For a review on somatostatin analogues, see: A. Janecka, M. Zubrzycka, T. Janecki, *J. Pept. Res.* **2001**, *58*, 91–107.

[6] Methyl groups in the γ^4 position of the first and in the γ^2 position of the second γ-amino acid do stabilize the turn (see ref. [1] for more details).

[7] M. Smrcina, P. Majer, E. Majerova, T. A. Guerassina, M. A. Eissenstat, *Tetrahedron* **1997**, *53*, 12867–12874; S. Hanessian, R. Schaum, *Tetrahedron Lett.* **1997**, *38*, 163–166; T. Hintermann, K. Gademann, B. Jaun, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 983–1002.

[8] The choice of a 2-naphthylacetyl group at the *N*-terminus was inspired by previous observations: The naphthyl group may be considered to mimic the benzyl side chain of a Phe residue, which precedes the Trp in the peptide chain of somatostatin: S. J. Hocart, R. Jain, W. A. Murphy, J. E. Taylor, B. Morgan, D. H. Coy, *J. Med. Chem.* **1998**, *41*, 1146–1154; A. J. Sowers, A. A. Virgilio, A. Rosenquist, W. Feniuk, J. A. Ellman, *J. Am. Chem. Soc.* **1999**, *121*, 1817–1825.

[9] B. H. Arison, R. Hirschmann, D. F. Veber, *Bioorg. Chem.* **1978**, *7*, 447–451; R. M. Freidinger, D. S. Perlow, W. C. Randall, R. Saperstein, B. H. Arison, D. F. Veber, *Int. J. Pept. Protein Res.* **1984**, *23*, 142–150; C. Wynants, D. Tourwe, W. Kazmierski, V. J. Hruby, G. Van Binst, *Eur. J. Biochem.* **1989**, *185*, 371.

[10] J. P. Hannon, C. Petrucci, D. Fehlmann, C. Viollet, J. Epelbaum, D. Hoyer, *Neuropharmacology* **2002**, *42*, 396–413. CCL-39 stands for Chinese hamster lung fibroblast cells No. 39. $[^{125}I]$LTT-SRIF$_{28}$ is a somatostatin dimer containing three mutations (Leu^8, D-Trp^{22}, ^{125}I-Tyr^{11}), and SRIF is the acronym for somatostatin release inhibiting factor, 28 refers to the numbere of amino acids in the physiologically active form of the hormone.

[11] J. Franckenpohl, P. I. Arvidsson, J. V. Schreiber, D. Seebach, *ChemBioChem* **2001**, *2*, 445–455.

Controlled Electropolymerization

Liquid-Crystal Templating of Conducting Polymers**

*James F. Hulvat and Samuel I. Stupp**

In organic electronics, conducting polymers have a number of advantages over small molecules, particularly because of their stability, mechanical properties, and ease of processing.[1,2] However, performance of some conducting polymers is limited by their high degree of disorder.[3,4] Molecular ordering improves carrier mobility in organic field-effect transistors and enhances charge injection in organic light-emitting diodes (OLEDs).[3] For this reason, vapor-sublimated crystalline films of small molecules are often used,[3] but alternative strategies to obtain molecular ordering would reduce cost and simplify fabrication of organic electronic devices. One possible way to achieve this is through molecular self-organization. Toward this goal we developed an aqueous, low-temperature technique for preparing conducting polymer films in a self-organized template.

Films of poly(3,4-ethyldioxythiophene) (PEDOT) are commonly used as hole injection layers in OLEDs. PEDOT can be polymerized in organic solvents or in an aqueous suspension with a soluble copolymer or surfactant, leading to amorphous films.[6] We have studied here the formation of PEDOT films by electropolymerization within a liquid crystalline template.

The well-known hexagonal (H1) lyotropic liquid crystal (LC) consists of cylindrical hydrophobic cores parallel to one another and separated by a hydrophilic continuum (see Figure 1 in the Supporting Information).[7] LCs have been used by us and others to template inorganic minerals as well as in the formation of mesoporous silica.[8] With regard to conducting polymers, template chemistry has been used to incorporate chains within the channels of mesoporous silica[9] or in aqueous channels of an inverse hexagonal LC.[10] These approaches, however, are limited to soluble, chemically polymerized polymers or to water-soluble monomers such as aniline or pyrrole. Our approach described here is novel in two key respects. First, polymerization occurs in the hydrophobic domain of the LC, allowing use of less polar

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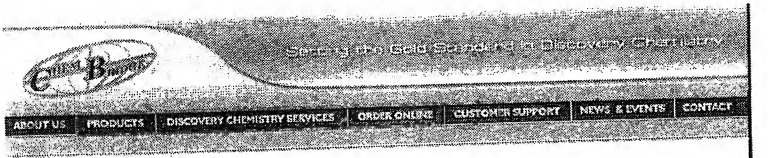
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

REFERENCE 2



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Company Overview

ChemBridge Corporation is a privately held US company founded in Chicago in 1993 and relocated to San Diego in 1997. ChemBridge has European offices in the UK, a marketing agency in Japan, and operates a large, state-of-the-art offshore chemistry research site in Moscow, Russia with over 300 employees, including 150 chemists (60 Ph.D., 80 M.S.).

ChemBridge offers an extensive portfolio of advanced discovery chemistry products and contract research services. These include premium quality hand-crafted or parallel-synthesized screening libraries, as well as exclusive customized discovery chemistry research services, which is currently the company's largest and fastest growing business division. In many cases such services are provided by ChemBridge Corporation jointly with ChemBridge Research Laboratories, Inc. (CRL). CRL is our affiliated San Diego based medicinal chemistry research and drug discovery company. CRL/ChemBridge combination provides our clients with the best blend of complementary advantages of both our US and our offshore research sites.

Over its history, ChemBridge has continuously set the industrial benchmark in innovations, quality and deliverability. This is the reason that the company is recognized as "The Gold Standard in Discovery Chemistry Outsourcing". ChemBridge has also built many long-term trust-based business relationships as well as an impeccable reputation with its client base. Over 400 pharmaceutical and biotech companies and universities worldwide have taken advantage of the access to ChemBridge's growing stock library of 600,000 diverse drug-like small molecule compounds. A number of leading pharmaceutical and biotech companies benefit from both the expertise and the dedication of ChemBridge's scientists that are involved in exclusive discovery chemistry research projects. The two largest research collaborations are with Pfizer, Inc., and Merck & Co, Inc., which together involve over 100 ChemBridge and CRL chemists. Recently, both alliances have been substantially expanded and extended based on a consistent track record of out-performing goals.

In 2000, ChemBridge Corporation formed an independent San Diego based spin-off company ChemBridge Research Laboratories, Inc. (CRL), www.chembridgeresearch.com. Currently CRL has a staff of 50 scientists (30 Ph.D. chemists) and a strong management team with significant research experience in the pharmaceutical industry. CRL focuses on the most value-added medicinal chemistry services and collaborations, as well as on its own proprietary drug discovery programs that currently

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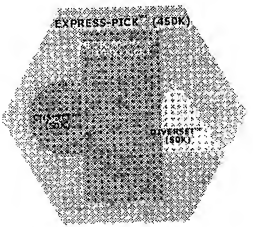

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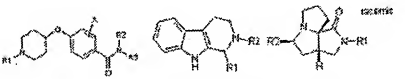
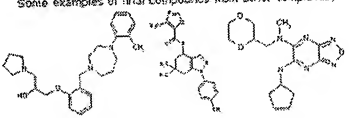


Some examples of final compounds from other templates:



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Specifications:

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Specifications:

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- Samples can be ordered on a per plate basis; typically one template scheme per plate
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- CDA required to view structures

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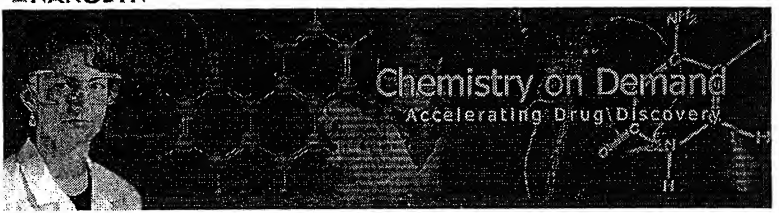

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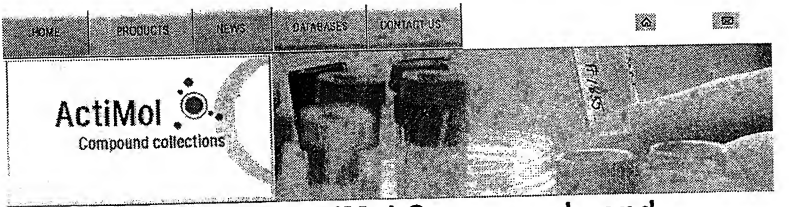

ActiMol Compounds and Compound Collections

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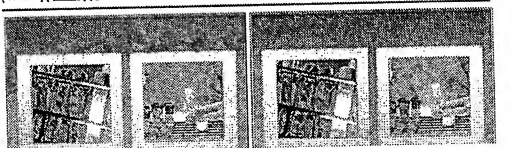

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. Serial No.: 10/648,089	Attorney Docket No.: 09820.286
Filing Date: August 26, 2003	Group Art Unit: 1654
Applicant(s): Gellman et al.	Examiner: Kosar

Title: HETEROGENEOUS FOLDAMERS CONTAINING α-, β-, AND/OR γ-AMINO ACIDS

RULE 132 DECLARATION OF SAMUEL H. GELLMAN

Mail Stop: **AMENDMENT**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
VIA FAX: 571-273-8300

To the Commissioner:

I, Samuel H. Gellman, do state and declare as follows:

1. I am a co-inventor of the invention described and claimed in the above-identified patent application. Thus, I am intimately familiar with the above-referenced patent application. I am knowledgeable regarding the synthesis, characterization, and uses of peptide mimetic compounds generally, and compounds containing α-amino acid, β-amino acid, and γ-amino acid residues in particular. I have authored or co-authored approximately 130 peer-reviewed papers in peptide mimetics and related fields. I received an A.B. degree in chemistry from Harvard University in 1981, and a Ph.D. degree in chemistry from Columbia University in 1986. After receiving my graduate degree, I worked as a post-doctoral fellow at the California Institute of Technology. Since 1987, I have been a professor of chemistry at the University of Wisconsin-Madison. I was very recently awarded the 2007 Ralph F. Hirschmann Award in Peptide Chemistry (for outstanding achievements in the chemistry, biochemistry, and biophysics of peptides). The award is sponsored by Merck Research Laboratories, and is presented by the American Chemical Society.

2. All of the experiments described in this declaration were performed by me, or at my direction and supervision. All of the experiments described in this declaration were performed in the United States of America.

3. The purpose of this declaration is two-fold. The first purpose of this declaration is to show that the *utility* of the compounds as described and claimed in the above-referenced application is well-established and well-understood to a chemist of ordinary skill in the field of peptide mimetics. The second purpose of this declaration is to show that the utility articulated in the application as filed is specific to the claimed compounds, is credible, and is concrete.

4. Regarding the first purpose of this declaration (that the *utility* of the compounds claimed in the application is established and well-understood to a chemist of ordinary skill), the application as filed specifically indicates that the compounds are useful as peptide mimetics that are not easily degraded by the action of proteolytic enzymes and that are capable of interrupting protein-protein interactions. This utility arises because the claimed compounds adopt stable secondary conformation and contain two or more unnatural β-amino acid residues and/or γ-amino acid residues in their backbones (thereby making them unsuitable as substrates for many protein-degrading enzymes). In particular, the passage spanning pages 19 and 20 of the application as filed succinctly sums up the major utility of the claimed compounds:

> The subject compounds find use as peptide mimetics that are not easily degraded by the action of proteolytic enzymes. Thus, the cyclically-constrained peptides of the present invention can be used as probes to explore protein-protein interactions. Because the compounds of the present invention are cyclically-constrained, they are more restricted conformationally than their strictly α-polypeptide counterparts. The compounds can be labeled and tracked throughout any given reaction. The effect the compound has on any given reaction provides valuable information on either or both of the kinetics and/or thermodynamics of the reaction being studied. Such reactions can be performed in vitro, in vivo, and ex vivo.
>
> Libraries of the subject compounds can also be prepared by automated means, thus providing access to a huge database which can be used as a tool to test, for example, potentially biologically-active agents.
>
> One highly useful aspect of the invention is that because the backbone is heterogenous, a portion of the residues, such as the α-amino acids, provide functional diversity (thus allowing many different types of reactions in many different types of environments to be explored), while the cyclically-constrained residues provide conformational specificity and stability. For example, massive diversity can be obtained using commercially-available α-amino acids as building

blocks, while structural rigidity is conferred by using only a single type of rigidified (i.e., cyclically-constrained) β- or γ-amino acid.

With particular focus on protein-protein interactions, it has long been a goal of biological scientists to disrupt specific protein-protein interactions as a means to explore the nature of the interaction. This goal has proven difficult to achieve using traditional small molecules. Binding size is likely part of the problem. Protein-protein complexes generally involve relatively large molecular surfaces. This makes it difficult for a small molecule to bind competitively at such a site. The present compounds, however, are polyamides and can be quite large. Thus, as a class, these compounds, individually and in the form of large libraries of compounds, are much better suited for probing protein-protein interactions than are small molecules. Additionally, the conformations of the subject compounds are periodic; the conformations can be extended simply by adding additional monomers to the polypeptide. Thus, the present compounds can be fabricated as relatively small skeletons or as very large skeletons, the size being dictated, at least in part, by the size of the binding site to be studied.

5. The *utility* noted in the above-quoted passage is well-established and well-understood to a chemist of ordinary skill in the field of peptide mimetics. In short, fabricating a large library of different compounds, in which every compound in the library shares some fundamental feature, is well known in the art. In the present claims, the compounds share the feature of having two or more unnatural, cyclically-constrained amino acid residues, either β-amino acid residues or γ-amino acid residues. The well-known utility of the specific compounds claimed, as well as related compounds, is that they mimic the pharmacological properties of natural peptides. However, because the claimed compounds contain unnatural amino acid residues, they are less prone to enzymatic degradation by proteases and peptidases. Thus, the specific chemical structure of the claimed compounds gives them a correspondingly specifc and substantial function to interrupt protein-protein interactions. This utility is reflected in both the scientific literature and by the commercial entities that construct and sell similar libraries.

6. In the scientific literature, there is a published paper that appeared contemporaneously to the filing date of the present application: Seebach et al. (2003) "Design and Synthesis of γ-Dipeptide Derivatives with Submicromolar Affinities for Human Somatostatin Receptors," *Angew. Chem. Int. Ed.*, 42(7):776-778, attached hereto as Exhibit A. The Seebach et al. paper describes related, unnatural γ-amino acid dipeptides polypeptide mimetics. The paper was submitted to the publisher on September 25, 2002 (prior the actual filing date of the present application), but after the earliest claimed priority date of the present application (August 26, 2002). The compounds described

by Seebach et al. are slightly smaller than those presently claimed (dipeptides vs. tetrapeptides), but are otherwise closely related to the present compounds in that Seebach's compounds are constructed of γ-amino acids. Most telling for purposes of this declaration is that Seebach's γ-amino acid dipeptide was made and tested for its ability to block the binding between somatostatin and a receptor protein. This is the same utility noted in the present application as filed. More specifically, Seebach and his co-workers made a γ-amino dipeptide and then tested it against five different human somatostatin receptors (hsst1, through hsst5). See the very first paragraph of the Seebach et al. paper. It is also noteworthy that Seebach et al. did not rationally design the dipeptide described in the paper. The dipeptide described by Seebach et al. was fabricated arbitrarily, from readily available, commercial starting materials. See page 776 the Seebach et al. paper. The discussion at the left-hand column of 777 of Seebach et al. and continuing to the top of the right-hand column, however, indicates that Seebach et al. also found evidence that the compound they tested adopts distinct and stable secondary structure. Seebach et al. describe their results as "confirmative, surprising, and promising." See page 777, right-hand column, second full paragraph. The significance of Seebach et al's work to the present application is that the Seebach et al. paper clearly shows that compounds falling within the same class as those now claimed have a utility that is well-known to peptide chemists; namely to mimic natural peptides using unnatural polypeptide-containing compounds. The utility evidenced in the Seebach et al. paper is the same utility articulated in the present application.

7. Similarly, see U.S. Patent 6,958,384, of which I am a co-inventor. This issued U.S. Patent discloses the same utility as disclosed in the present application. The compounds described in U.S. Patent No. 6,958,384 are oligomers made entirely from of γ-amino acids, at least one of which is cyclically-constrained. Thus, the compounds described in the issued patent are structurally similar to the presently claimed oligomers, which contain non-constrained and cyclically-constrained residues.

8. As further evidence of the well-known and well-established utility of the claimed compounds, I note that chemical compound libraries (such as the compounds now claimed) are articles of commerce. A chemist clearly understands that such compound libraries are useful. See Exhibits B, C, and D, attached hereto and incorporated herein. These three Exhibits are screen shots

from the chemical library companies ChemBridge Corporation (San Diego, California), NanoSyn Corporation (Menlo Park, California), and ActiMol (Newark, Delaware). All three companies sell chemical libraries for drug discovery and other purposes. One of the three, ChemBridge Corporation, has been making and selling chemical libraries in the United States since 1993. Exhibits B, C, and D clearly demonstrate a well-established utility for chemical libraries, and also demonstrate that that utility is understood by chemists. An ordinarily skilled chemist would not pay good money for a chemical library that has no use.

9. Regarding the second purpose of this declaration, namely that the present compounds have a specific, credible, and concrete utility, the Examiner stated (at page 5 of the Office Action dated May 5, 2006), that the Exhibits submitted earlier with respect to utility were not sufficiently probative evidence because the Exhibits "did not discuss the particulars of the present invention, *e.g.*, examples of the instantly claimed compounds, but rather generalizations on peptide libraries." I thus submit the following examples to show that the utility asserted in the patent application is specific to the presently claimed compounds and that undue experimentation **is not required** to identify or to confirm this utility. Thus, the utility of the compounds as articulated is specific, credible, tangible and easily confirmed by a chemist of ordinary skill.

10. To provide some background to the examples that follow, molecules that bind to specific protein surface sites are of fundamental interest (*e.g.*, from the perspective of molecular recognition) and of practical interest (*e.g.*, from the perspective of medicine). Because they can bind to specific protein surfaces, such molecules can disrupt specific protein-protein interactions, which are frequently associated with human diseases. Traditional "small molecule" approaches, very successful for enzyme inhibition, have been less productive for generating protein-protein interaction antagonists,[1] although some recent achievements are very impressive.[2] I and others have used unnatural oligomers with discrete folding propensities ("foldamers") to provide a rational basis (*i.e.*, a non-random basis) to make molecules that block protein-protein interactions.[3] In the examples that follow, my co-workers and I explored this specific utility in the context of Bcl-x_L/BH3 domain

interactions, a system that is attractive because there is a considerable amount of structural information on the system.[4]

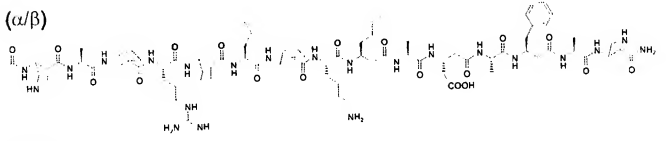
11. The results of the experiments show that foldamer-based designs can provide tight-binding ligands for a large protein-recognition site (K_i for compound **4** = 0.7 nM). The tight binding of chimeric ($\alpha/\beta+\alpha$)-peptides to Bcl-x_L suggests that combining different foldamer scaffolds is also an effective (and perhaps general) strategy for protein ligand design.

12. Interactions within the Bcl-2 protein family control the fate of a cell in response to cytotoxic stimuli. In many cancers, anti-apoptotic Bcl-2 proteins such as Bcl-x_L are overexpressed and protect malignant cells from death (apoptosis) by direct interaction with pro-apoptotic proteins such as Bak and Bad.[5] Thus, inhibitors of the Bcl-x_L/Bak interaction are therapeutically useful. A 16-residue peptide from the BH3 domain of Bak binds to a hydrophobic groove on Bcl-x_L as an α-helix, burying four hydrophobic side chains (Val-74, Leu-78, Ile-81 and Ile-84).[6] Many small molecule ligands for the BH3-recognition domain have been described. Most have only modest activity (IC_{50} values in competition binding assays typically > 1 μM),[7] perhaps because of the large surfaces buried in the Bcl-x_L/Bak 16-mer complex; however, a potent small molecule has very recently been reported.[2e] Numerous medium-length α-peptides (16-32 residues) have shown high affinity for Bcl-x_L.[8] As shown in the following paragraphs, compounds as claimed in the present application and that mimic the α-helical display of Bak side chains are a good source of Bcl-x_L/Bak interaction antagonists.[3c] Foldamers can be proteolytically stable,[9] a distinct advantage relative to α-peptide inhibitors.[9] The following experiments describe foldamers containing both α- and β-amino acid residues that compete effectively with the Bak 16-mer for binding to Bcl-x_L.

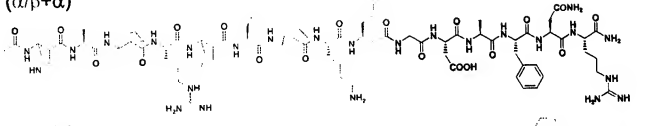
13. Our work focused on the 14/15-helix formed by α/β-peptides (oligomers with a 1:1 alternation of α- and β-amino acid residues along the backbone).[10] My co-workers and I designed new Bcl-x_L ligand candidates based on this secondary structure. These compounds fall within the scope of the present claims. The designs based on the 14/15-helix displayed significant activity in fluorescense polarization FP assays (although these α/β-peptides were not as effective as α-peptides

corresponding to natural BH3 domain sequences). For example, α/β-peptide 15-mer **1** displayed IC_{50} = 40 μM (K_i = 1.5 μM),[11] while IC_{50} = 0.67 μM (K_i = 0.025 μM) for the unlabeled Bak 16-mer (Figure 1).[14] In **1** and related α/β-peptide designs, Leu-6 is intended to play the role of the Leu residue conserved in all BH3 domains reported to date (*e.g.*, Leu-78 of Bak).[6] We speculate that ACPC-3, β^3-homonorleucine-9 (β^3-hNle-9) and β^3-hPhe-13 of **1** also contribute to the hydrophobic surface required for binding to the BH3-recognition cleft of Bcl-x_L. Arg-4 and Asp-11 of **1** may be involved in electrostatic interactions with residues on the edge of the Bcl-x_L cleft, as proposed for analogous residues in the Bak 16-mer.[6]

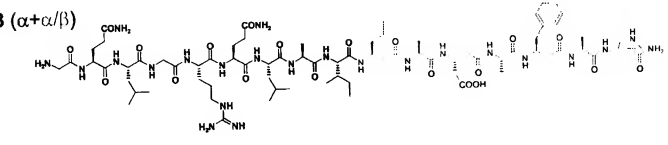
1 (α/β)



2 (α/β+α)



3 (α+α/β)



14. We then examined chimeric oligomers in which either the N-terminal portion or the C-terminal portion of compound **1** is replaced by an α-amino acid segment based on an α-peptide known to bind tightly to Bcl-x_L. For example, (α/β+α) oligomer **2** contains the first nine residues of α/β-peptide **1**, but the last six α-residues are related to the C-terminal segment of the Bak 16-mer, with Phe-13 of **2** intended to correspond to Ile-84 of Bak. In (α+α/β) oligomer **3**, the first nine residues correspond to positions 72-81 of Bak with Val-74 replaced by Leu; the final seven residues correspond to the C-terminal portion of α/β-peptide **1**. These complementary chimeric analogues of **1** show very different activities in the FP assay: for (α/β+α) oligomer **2** IC_{50} = 0.059 μM (K_i = 0.0019 μM), while for (α+α/β) oligomer **3** IC_{50} > 700 μM (see Figure 1). Thus, **2** is 10-fold more potent than the Bak 16-mer. This result indicates that the 14/15-helical α/β-peptide scaffold is well-suited to occupy at least a portion of the BH3-recognition cleft on Bcl-x_L.

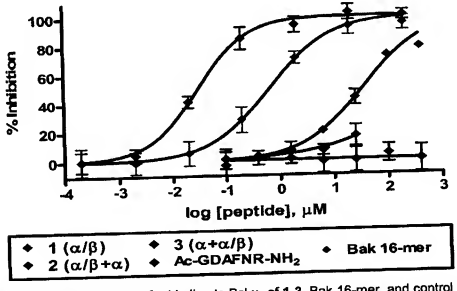


Figure 1. Competition FP data for binding to Bcl-x_L of **1**-**3**, Bak 16-mer, and control hexa-α-peptide Ac-GDAFNR-NH_2. A fluorophore-labeled Bak 16-mer peptide was used as the displaced fluorescent probe.

15. Several control studies were conducted with ($\alpha/\beta+\alpha$) oligomer **2** and related compounds. The hexa-α-peptide corresponding to the C-terminal segment of **2** (Ac-GDAFNR-NH_2) at 500 μM displayed no interaction with Bcl-x_L in the FP assay (see Figure 1). (Thus, the α-peptide segment of chimeric oligomer **2** is probably not the dominant contributor to Bcl-x_L binding affinity.[12])

16. Binding of **2** to ^{15}N-labeled Bcl-x_L was examined via [^{1}H,^{15}N]-HSQC NMR measurements (see Figure 2). Most of the Bcl-x_L amide N-H cross peaks were significantly shifted upon addition of 50 μM **2** to 100 μM Bcl-x_L (Figure 2A). The pattern of shifts and resonance broadening induced by addition of **2** are comparable to the effects induced by addition of the Bak 16-mer α-peptide (see Figure 2B).

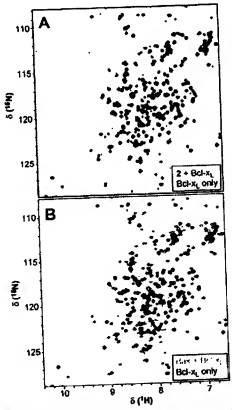


Figure 2. HSQC NMR binding assays. (A) Overlay of ^{15}N-Bcl-x_L spectra in presence (red) and absence (black) of ($\alpha/\beta+\alpha$)-peptide **2**. (B) Overlay of ^{15}N-Bcl-x_L spectra in presence (green) and absence of the Bak 16-

17. A fluorescein-labeled derivative of **2** (**Flu-2**) was prepared to compare binding to Bcl-x_L with binding to unrelated proteins. Direct FP titration of 50 nM **Flu-2** with protein indicates K_d = 0.014 μM for **Flu-2** binding to Bcl-x_L (Figure 3). In contrast, no binding to bovine γ-globulin (BGG) could be detected at 500 μM BGG, and the onset of binding to bovine serum albumin (BSA) occurred above 10 μM BSA (Figure 3). Thus, binding of **Flu-2** to BGG or BSA is at least 10^3-fold weaker than binding to Bcl-x_L. Both BGG and BSA are promiscuous receptors for hydrophobic ligands.[13] This result is highly significant because the failure of **Flu-2** to bind tightly to either of these proteins indicates that the affinity of **2** for Bcl-x_L is not simply the result of its hydrophobicity, but instead reflects complementarity to the BH3-recognition cleft. As a further test of such complementarity, we compared ($\alpha/\beta+\alpha$) oligomer **4** and its enantiomer in the competition FP assay (Figure 4). Oligomer **4** is an isomer of **2** in which β^3-hNle-9 has been replaced by β^3-hLeu; this small change leads to slightly improved affinity for Bcl-x_L (IC_{50} = 0.029 μM, K_i = 0.0007 μM for **4**). The enantiomer of **4**, however, displays very low affinity for Bcl-x_L (IC_{50} > 1000 μM). Again, this result is significant because it indicates that the affinity shown is not simply the result of the hydrophobicity of **4**.

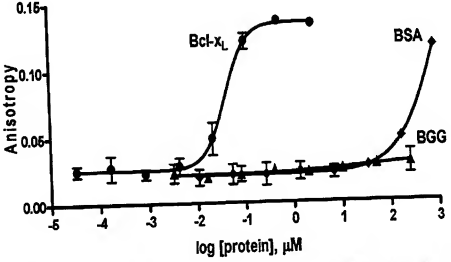
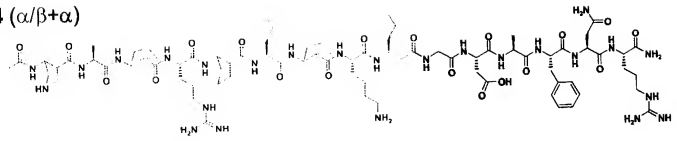


Figure 3. Direct FP titration of fluorescein-labeled ($\alpha/\beta+\alpha$)-peptide **Flu-2** with Bcl-x_L, BSA, and BGG.

4 ($\alpha/\beta+\alpha$)



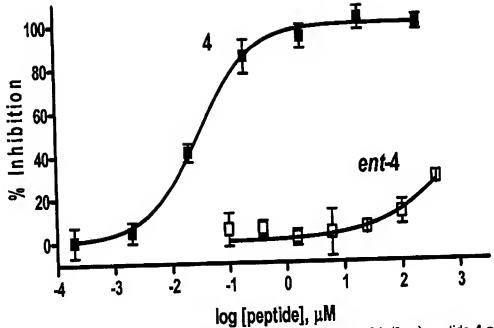


Figure 4. Competition FP binding assay for binding to Bcl-x_L of ($\alpha/\beta+\alpha$)-peptide **4** and its enantiomer.

18. The folding of ($\alpha/\beta+\alpha$) oligomer **5** in CD_3OH was examined by 2D NMR.[14] Compound **5** has two modifications relative to **2** (Ala-2 → Lys and Lys-8 → Ile), which moderately diminish binding to the BH3-recognition cleft of Bcl-x_L (IC_{50} = 0.40 μM). Good dispersion of ^1H resonances was observed for **5**, which allowed assignment of many NOEs between backbone protons. Numerous i,i+3 NOEs were observed along the entire length of **5** (Figure 5). Of particular importance are the three α-residue $H_\alpha(i)$ → β-residue $H_\alpha(i+3)$ NOEs in the α/β-peptide segment of **5**. This NOE pattern is predicted for the 14/15-helix but not for the 11-helix.[10] In contrast, α-residue $H_\alpha(i)$ → α-residue NH(i+2) NOEs are predicted for the 11-helix but not for the 14/15-helix,[12] and none of these NOEs is observed for **5**. Thus, the NMR data suggest that **5** has a substantial propensity to adopt the 14/15-helical secondary structure in its N-terminal region, a propensity that is likely to be manifested also by closely related molecules such as **2**. Interestingly, the i,i+3 NOEs involving the C-terminal α-peptide region of **5** suggest that the 14/15-helical α/β-segment can nucleate helix formation in the short α-peptide segment.

Figure 5. NOEs observed for oligomer **5** in CD_3OH. NOEs consistent with the 14/15-helix only (blue arcs), both the 11- and 14/15-helix (black solid arcs), and α-helix (red arcs). Ambiguously-assigned NOEs are represented by dotted arcs.

19. The results of the experiments are significant because the reflect the exact utility that is stated within the patent application. The subject compounds mimic natural protein conformations in solution, but are significantly protected from proteolytic degradation by proteases and peptidases. The compounds are useful probes in the study of chemical and enzymatic interactions involving natural proteins. As shown above, compounds according to the present invention provide tight-binding ligands for a large protein-recognition site (K_i for compound **4** = 0.7 nM). The tight binding of chimeric ($\alpha/\beta+\alpha$)-peptides to Bcl-x_L indicates that combining different foldamer scaffolds is also an effective strategy for protein ligand design.

20. Another biological application of heterogeneous α/β-peptide foldamers involves inhibition of viral infection. My colleagues and I have designed α/β-peptides intended to block infection of human fibroblast cells with human cytomegalovirus (CMV), which is a source of human disease. Data for a few examples is shown in Figure 6. (The structures of the compounds tested are shown in Figure 7.) In this assay, the virus expresses green fluorescent protein (GFP), so we tracked green fluorescence to learn how many cells have been infected. For compounds VI-139 and VI-145, the data show significant inhibition of viral infection when the α/β-peptide is present at 50 μM. These results show that α/β-peptides can block CMV infection of target cells, which is a biomedically valuable, structure-specific property.

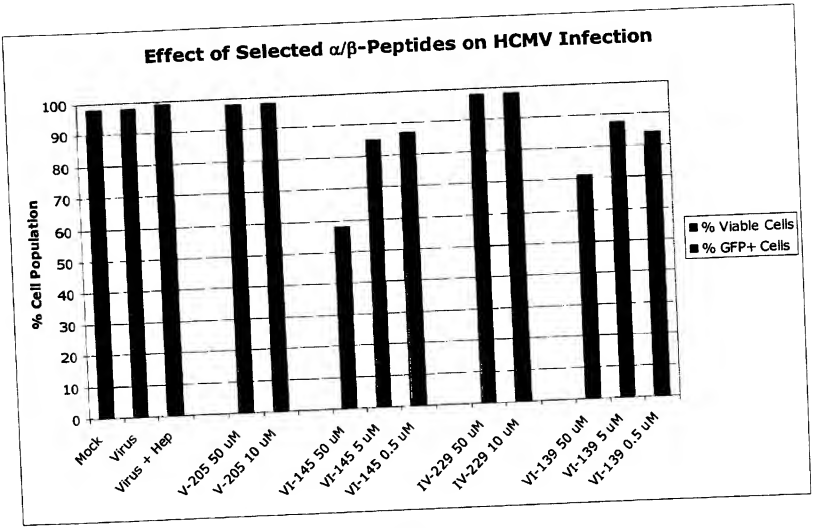
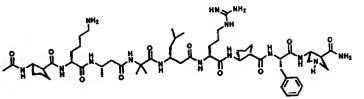
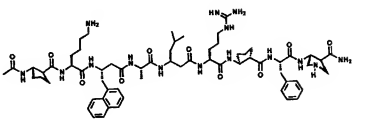

Effect of Selected α/β-Peptides on HCMV Infection
% Cell Population
100
90
80
70
60
50
40
30
20
10
0
Mock
Virus
Virus + Hep
V-205 50 uM
V-205 10 uM
VI-145 50 uM
VI-145 5 uM
VI-145 0.5 uM
IV-229 50 uM
IV-229 10 uM
VI-139 50 uM
VI-139 5 uM
VI-139 0.5 uM
% Viable Cells
% GFP+ Cells

Figure 6.

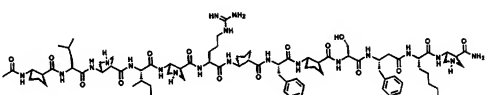
EPE-V-205



EPE-VI-145



EPE-IV-229



EPE-VI-139

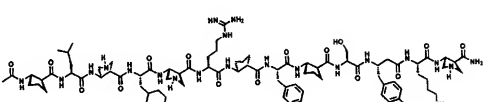


Figure 7.

21. I, Samuel H. Gellman, hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this Rule 132 Declaration is directed.

Samuel H. Gellman 2 Nov 06

Date

ENDNOTES

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(12) The α/β-peptide oligomer corresponding to residues 1-9 of chimeric oligomer **4**, an isomer of **2** (see text), has an IC_{50} of 120 μM, suggesting that the α/β portions of **2** and **4** target the BH3 binding site and contribute substantially to binding.

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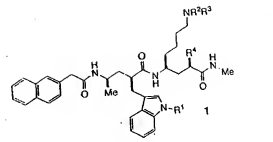
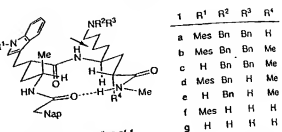
[16] We found that BRP-H is also a polymerization catalyst for other thiophenes, for example, 3-dodecyl-thiophene.
[17] *Handbook of Oligo- and Polythiophenes* (Ed.: D. Fichou), Wiley-VCH, Weinheim, 1999.
[18] D. Dini, F. Decker, F. Andreani, E. Salatelli, P. Hapiot, *Polymer* 2000, *41*, 6473.
[19] In the measurement setup an argon ion laser beam was focused by a 25× microscope objective to a 50 μm diameter spot on the sample. The fluorescence emission was imaged by a 50× objective on a CCD camera.
[20] No UV/Vis spectrum could be recorded from a single vesicle, nor from a suspension of vesicles, because of scattering problems.
[21] A 0.5 g L^{-1} solution of PS-PIAT dissolved in THF was injected into a 30 mg L^{-1} solution of CAL B enzymes; the final water/THF ratio was 12:1 (v/v). After the mixture was left for two days to equilibrate, it was dialyzed to dispose of all nonincluded enzymes.





	R^1	R^2	R^3	R^4
a	Mes	Bn	Bn	H
b	Mes	Bn	Bn	Me
c	H	Bn	Bn	Me
d	Mes	Bn	H	Me
e	H	Bn	H	Me
f	Mes	H	H	H
g	H	H	H	H

Expected conformation of **1**

Scheme 1. Structural formulae of the γ-peptides **1**. In the expected conformation of **1** the red arrow points to a CH_3 group of the $H_2N(CH_2)_4$ unit, which is placed inside the shielding cone of the aromatic indole ring. Mes = mesitylenesulfonyl, Bn = benzyl, Nap = naphthyl.

Figure 1. peptides field-shift

A ty its anal side ch inside t shifts b section shown signals 0.6 ppm tween chains, with a compc N-nap 1 exhi ion of 70000

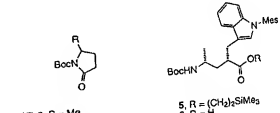
Somatostatin Mimics

Design and Synthesis of γ-Dipeptide Derivatives with Submicromolar Affinities for Human Somatostatin Receptors

Dieter Seebach, Laurent Schaeffer, Meinrad Brenner, and Daniel Hoyer*

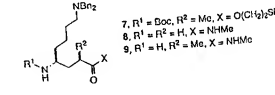
In a previous paper we have shown that simple *N*-acyl-γ-dipeptide amides that resemble a βII′ turn of an α-peptide can be designed to form a turn structure in solution (NMR) and in the solid state (X-ray).[1,2] To see whether such a turn could also be used to mimic a peptide, the biological activity of which rests upon a turn structure carrying functionalized side chains, we have now synthesized compounds **1a–g** (Scheme 1), with the side chain of tryptophan in the γ^2 position of the first and of lysine in the γ^4 position of the second γ-amino acid, and have tested their affinities for the human somatostatin receptors $hsst_{1-5}$.[3–6]

The synthesis of γ-dipeptide derivatives **1** commenced with the *N*-Boc-γ-lactams **2** and **3** (Boc = *tert*-butoxycarbonyl), readily available from the corresponding commercial (*R*)-Ala and (*S*)-Lys acids by known procedures.[1,7] Ring opening (with the Lys derivative after change of side-chain protection, →**4**), and esterification with $Me_3Si(CH_2)_2OH$ provided the (*R*)-Boc-γ^4-hhAla and Boc-γ^4-hhLys(Bn_2) esters, which were



(*R*)-**2**, R = Me
(*S*)-**3**, R = $(CH_2)_4NH$(2-Cl-Z)
(*S*)-**4**, R = $(CH_2)_4NBn_2$

5, R = $(CH_2)_2SiMe_3$
6, R = H



7, R^1 = Boc, R^2 = Me, X = $O(CH_2)_2SiMe_3$
8, R^1 = R^2 = H, X = NHMe
9, R^1 = H, R^2 = Me, X = NHMe

doubly deprotonated and alkylated with 1-mesitylenesulfonyl-3-bromomethylindole and MeI to give the *unlike* $\gamma^{2,4}$-amino acid derivatives **5** and **7**, respectively. The ester group in compound **5** with Trp side chain was cleaved (Bu_4NF, →**6**), and the lysine-derived esters were converted to the methylamides **8** and **9** without and with 2-methyl substitution, respectively (1. Bu_4NF, 2. $MeNH_2$, 3. F_3CCO_2H). Coupling of the two γ-amino acid derivatives (**6** + **8** and **6** + **9**), removal of the Boc groups, and acylation with 2-naphthylacetic acid[8] (4-methylmorpholine, 1-hydroxy-1*H*-benzotriazole, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) produced the side-chain-protected *N*-acyl-dipeptide amides **1a** and **1b**. Deprotection procedures ($MeSO_3H$, F_3CCO_2H, and Pd/C, H_2) led to the various partially or fully deprotected γ-dipeptide derivatives **1c–1g**. All compounds were purified and fully characterized by elemental analyses, specific optical rotations, circular dichroism (CD), IR, and NMR spectroscopy, and mass spectrometry.

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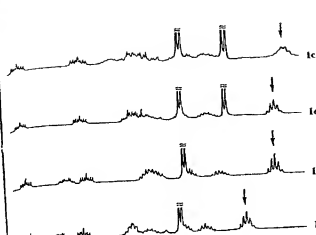

Figure 1. High-field part of the 500 MHz ^{1}H NMR spectra of the γ-dipeptides **1c**, **1e**, **1f**, and **1g** in CD_3OD. The red arrows point to high-field-shifted $N(CH_2)_4$ resonances.

A typical feature of the turn structure in somatostatin and its analogues is the juxtaposition of the tryptophan and lysine side chains, which places CH_2 groups of the $H_2N(CH_2)_4$ unit inside the shielding cone of the aromatic indole ring (NMR shifts between $\delta = 0.8$ and 0.3 ppm are observed).[9] High-field sections of the NMR spectra of four γ-dipeptide amides, shown in Figure 1, in which CH_2 signals appear between $\delta = 0.9$ and 0.6 ppm, confirm the proximity between the corresponding side chains, and are thus compatible with a turn conformation of these compounds. The CD spectra of the *N*-naphthylacetyl dipeptide amides 1 exhibit an intensive negative Cotton effect near 200 nm ([Θ] up to 70000 deg cm^2 dmol^{-1}), with a weaker and broader peak near 220 nm ([Θ] up to 30000 deg cm^2 dmol^{-1}) (Figure 2); this CD pattern may be taken as another piece of evidence for the presence of a secondary structure.

Probably the most stringent test of the γ-dipeptide structure is the affinity for somatostatin receptors. Binding affinities for the five cloned human receptors $hsst_{1-5}$, expressed in CCL-39 cell lines, were determined by displacement of [^{125}I]LTT-SRIF$_{28}$ from these receptor proteins.[10] While the fully protected γ-dipeptide **1d** binds to $hsst_1$ and $hsst_3$ with remarkable K_D values of 0.55 and 1.00 μM, respectively, the partially and the fully deprotected γ-dipeptide derivatives **1f** and **1g** bind to $hsst_3$ with K_D values of 0.51 and 0.87 μM, respectively (Table 1). Intriguingly, the highest affinities (**1d**/$hsst_1$, **1f**/$hsst_3$) are observed when the side chain functional groups (3-indolylmethyl and $(CH_2)_4NH_3^+$) are protected by bulky aromatic moieties (*N*-mesitylenesulfonyl and/or -benzyl)!

The results presented here are confirmative, surprising, and promising; they demonstrate that a 14-amino-acid cyclic disulfide hormone, somatostatin, can be mimicked by a simple, designed, low-molecular-weight, open-chain γ-dipeptide derivative (cf. **1g**) that contains only three amide bonds; they suggest that hitherto unknown hydrophobic pockets are present in the receptors ($hsst_1$, $hsst_3$, and $hsst_4$), which supposedly house the turn-bound Trp and Lys side chains (cf. **1c**, **1d**, **1f**); and they promise a potential of γ-peptides for the development of peptidase-resistant[11] peptidomimetic drugs.

Table 1: pK_D Values for γ-peptides **1b–1g** at the five hsst receptors expressed in CCL-39 cells and measured by radioligand binding assays with [^{125}I]LTT-SRIF$_{28}$ as radioligand.[9],[10]

Receptor	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	Octreotide[a]	SRIF$_{14}$[a]
$hsst_1$	5.47	6.06	6.26	5.61	5.98	4.73	6.45	9.08
$hsst_2$	< 5	< 5	5.17	< 5	5.01	2.81	9.11	10.06
$hsst_3$	5.53	5.89	6.00	5.73	5.67	5.42	8.60	9.67
$hsst_4$	4.67	5.74	5.92	5.66	5.79	5.44	5.76	8.39
$hsst_5$	4.49	5.01	5.87	5.14	6.29	6.06	7.31	9.01

[a] Submicromolar affinities are highlighted in red. [b] Sandostatin. [c] Somatostatin.

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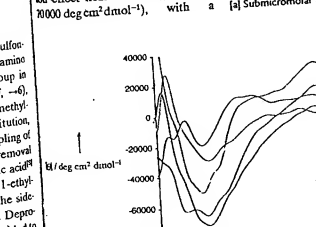

Figure 2. Nonnormalized CD spectra in MeOH (0.2 mM) of the γ-dipeptide derivatives **1b–1g**.

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Controlled Electropolymerization

Liquid-Crystal Templating of Conducting Polymers**

*James F. Hulvat and Samuel I. Stupp**

In organic electronics, conducting polymers have a number of advantages over small molecules, particularly because of their stability, mechanical properties, and ease of processing.[1,2] However, performance of some conducting polymers is limited by their high degree of disorder.[3,4] Molecular ordering improves carrier mobility in organic field-effect transistors and enhances charge injection in organic light-emitting diodes (OLEDs).[3] For this reason, vapor-sublimated crystalline films of small molecules are often used,[3] but alternative strategies to obtain molecular ordering would reduce cost and simplify fabrication of organic electronic devices. One possible way to achieve this is through molecular self-organization. Toward this goal we developed an aqueous, low-temperature technique for preparing conducting polymer films in a self-organized template.

Films of poly(3,4-ethylidioxythiophene) (PEDOT) are commonly used as hole injection layers in OLEDs. PEDOT can be polymerized in organic solvents or in an aqueous suspension with a soluble copolymer or surfactant, leading to amorphous films.[6] We have studied here the formation of PEDOT films by electropolymerization within a liquid crystalline template.

The well-known hexagonal (H1) lyotropic liquid crystal (LC) consists of cylindrical hydrophobic cores parallel to one another and separated by a hydrophilic continuum (see Figure 1 in the Supporting Information).[7] LCs have been used by us and others to template inorganic minerals as well as in the formation of mesoporous silica.[8] With regard to conducting polymers, template chemistry has been used to incorporate chains within the channels of mesoporous silica[9] or in aqueous channels of an inverse hexagonal LC.[10] These approaches, however, are limited to soluble, chemically polymerized polymers or to water-soluble monomers such as aniline or pyrrole. Our approach described here is novel in two key respects. First, polymerization occurs in the hydrophobic domain of the LC, allowing use of less polar

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

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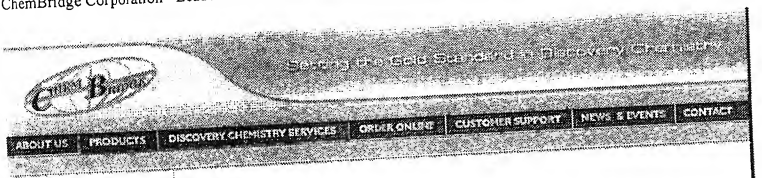

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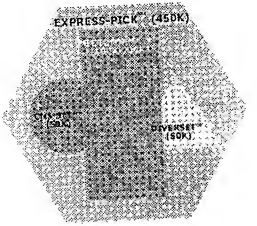

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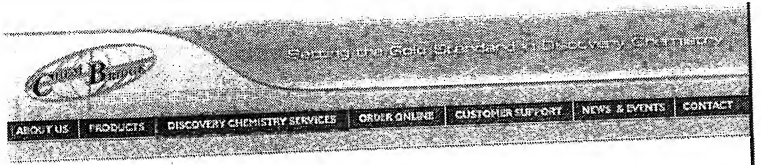

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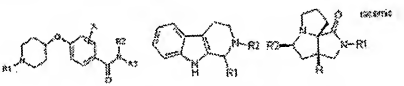

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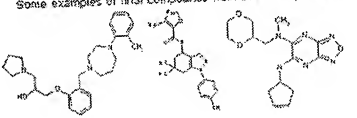

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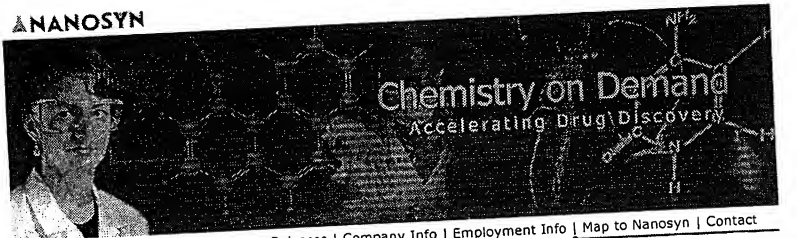

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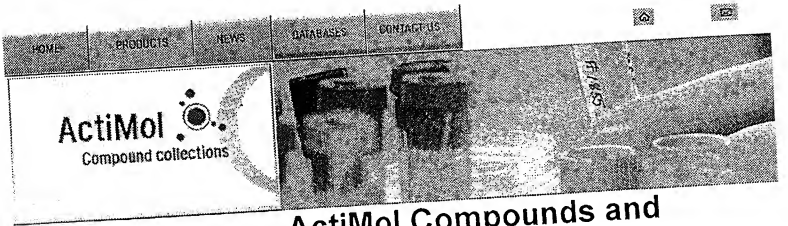

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(12) **United States Patent**
Gellman et al.

(10) **Patent No.: US 6,958,384 B2**
(45) **Date of Patent: Oct. 25, 2005**

(54) **POLYPEPTIDES CONTAINING γ-AMINO ACIDS**

(75) Inventors: **Samuel H. Gellman**, Madison, WI (US); **Matthew G. Woll**, Madison, WI (US); **Jonathan R. Lai**, Madison, WI (US); **Justin Murray**, Madison, WI (US)

(73) Assignee: **Wisconsin Alumni Research Foundation**, Madison, WI (US)

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(21) Appl. No.: **10/389,500**

(22) Filed: **Mar. 14, 2003**

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Related U.S. Application Data

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(51) **Int. Cl.**[7] **C07K 2/00**
(52) **U.S. Cl.** **530/332**; 530/300
(58) **Field of Search** 530/300, 332, 530/350

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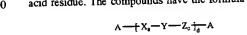
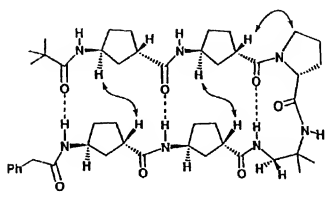
(57) **ABSTRACT**

Disclosed are polypeptide compounds containing at least one residue comprising a cyclically-constrained γ-amino acid residue. The compounds have the formula



where A is a hydrogen, hydroxy, amino- or carboxy-protecting group, Y is a single bond or a prolyl-containing linking group, and X and Y are γ-amino acid residues, provided that one of X or Y is a conformationally-restrained γ-amino acid residue, and "a," "c," and "d" are positive integers. The compounds find use as non-enzymatically degradable probes to mimic protein behavior in solution.

26 Claims, 3 Drawing Sheets



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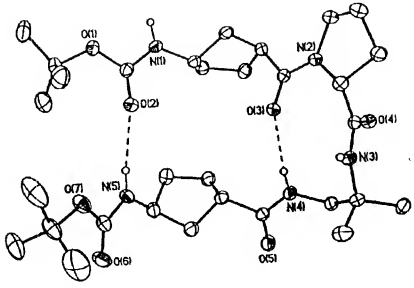


FIG. 1

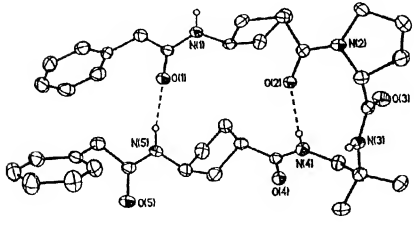


FIG. 2

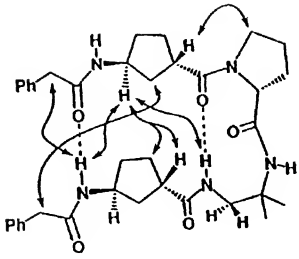


FIG.3

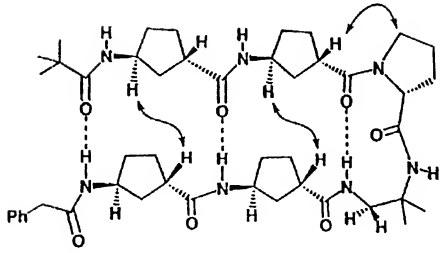


FIG. 4

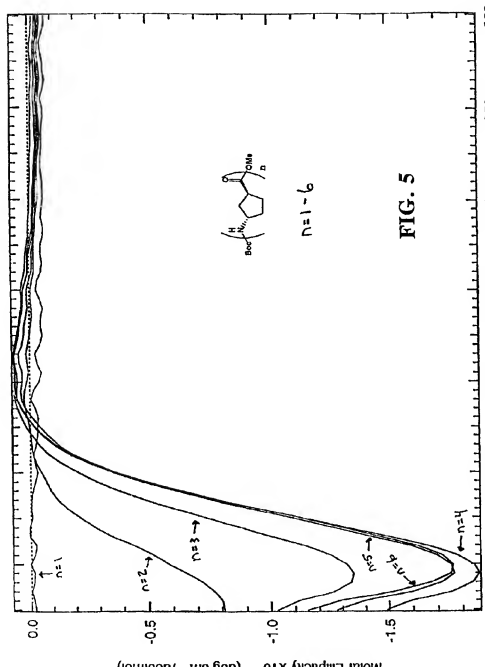

Molar Ellipticity x10 ^{4} (deg cm ^{2}/decimol)
0.0
-0.5
-1.0
-1.5
200
210
220
230
240
250
260
Wavelength (nm)
n=1
n=2
n=3
n=4
n=5
n=6
n=1-6

FIG. 5

POLYPEPTIDES CONTAINING γ-AMINO ACIDS

Priority is hereby claimed to provisional application Ser. No. 60/364,406, filed Mar. 15, 2002, and incorporated herein by reference.

FEDERAL FUNDING STATEMENT

This invention was made with United States government support awarded by the following agencies: NSF 9820952. The United States has certain rights in this invention.

FIELD OF THE INVENTION

The invention is directed to polypeptides comprising cyclically-constrained γ-amino acids. These novel, unnatural peptidomimetics are resistant or wholly immune to peptidase and protease degradation and are conformationally restrained. Thus, they are useful as tools to model peptide and protein conformations in aqueous solutions. The compounds are also useful as non-enzymatically degradable probes to mimic protein behavior in solution.

DESCRIPTION OF THE RELATED ART

Chemists have long sought to extrapolate the power of biological catalysis and recognition to synthetic systems. These efforts have focused largely on low molecular weight catalysts and receptors. Most biological systems, however, rely almost exclusively on large polymers such as proteins and RNA to perform complex chemical functions. Predicting and modeling the solution-phase behavior of these large molecules has also been an on-going and sustained effort conducted by many groups.

Proteins and RNA are unique in their ability to adopt compact, well-ordered conformations. These two biopolymers are unique also because they can perform complex chemical operations (e.g., catalysis, highly selective recognition, etc.). Folding is linked to function in both proteins and RNA because the creation of an "active site" requires proper positioning of reactive groups. Consequently, there has been a long-felt need to identify synthetic polymer backbones which display discrete and predictable folding propensities (hereinafter referred to as "foldamers") to mimic natural biological systems. Such backbones will provide molecular "tools" to probe the functionality of large-molecule interactions (e.g. protein-protein and protein-RNA interactions). Insofar as these unnatural backbones are resistant to the action of proteases and peptidases, they are useful as probes having constrained conformational flexibility. Whereas a naturally occurring, α-amino acid probe will be readily degraded by any number of proteases and peptidases, foldamers are not.

Much work on β-amino acids and peptides synthesized therefrom has been performed by a group led by Dieter Seebach in Zurich, Switzerland. See, for example, Seebach et al. (1996)[a] and Seebach et al. (1996)[b]. In the first of these two papers Seebach et al. describe the synthesis and characterization of a β-hexapeptide, namely (H-β-HVal-β-HAla-β-HLeu)$_2$-OH. Interestingly, this paper specifically notes that prior art reports on the structure of β-peptides have been contradictory and "partially controversial." In the second paper, Seebach et al. explore the secondary structure of the above-noted β-hexapeptide and the effects of residue variation on the secondary structure.

Dado and Gellman (1994) describe intramolecular hydrogen bonding in derivatives of β-alanine and γ-amino butyric acid. This paper postulates that β-peptides will fold in manners similar to α-amino acid polymers if intramolecular hydrogen bonding between nearest neighbor amide groups on the polymer backbone is not favored.

Suhara et al. (1996) report a polysaccharide analog of a β-peptide in which D-glycocylamine derivatives are linked to each other via a C-1 β-carboxylate and a C-2 α-amino group. This class of compounds has been given the trivial name "carbopeptoids."

Hamuro et al. (1999) describe antibacterial compositions containing β-peptides having a repeating 3-peptide residue motif. The compounds described are: Fmoc-(β^3-HVal-β^3-HLys-β^3-HLeu)$_n$-OH (n=2–4); H-(β^3-HVal-β^3-HLys-β^3-HLeu)$_n$-OH (n=2–4); and H-(β^3-HLeu-β^3-HLys-β^3-HLeu)$_n$-OH (n=2–6). While these β-peptides are described as being antibacterial, they are also hemolytic at concentrations near the effective antibacterial concentrations, thus limiting their utility as medicaments.

As noted above, the interest in foldamers stems in part from their resistance to enzymatic degradation. They are also interesting molecules because of their conformational behavior. The elucidation of foldamers having discrete conformational propensities akin to those of natural proteins has led to numerous recent explorations of peptides constructed from β-, γ-, or δ-amino acids. For recent reviews, see, for example, Seebach & Matthews (1997), Gellman (1998)[c] and Degrado et al. (1999). γ-Peptides containing residues bearing γ-substitution or α,γ-disubstitution or α,β,γ-trisubstitution have been shown to adopt a helical conformation defined by a 14-member turn that is stabilized by C=O(i)→NH(i+3) hydrogen bonds. See Hintermann et al. (1998) and Hanessian et al. (1998). Hanessian et al. (1999) have reported reverse turn formation by a γ-peptide built from α,γ-disubstituted residues having a stereochemistry that is different from that leading to helical folding.

In the hairpin loop architecture, found in natural proteins, two strands of the amino acid backbone of the molecule are connected by a short loop. The hairpin loop is essential for creating small increments of β-sheet secondary structure in conventional peptides. See Gellman (1998)[b] and Lacroix et al. (1999). Formation of β-sheet secondary structure requires non-covalent attraction between the strand segments, as well as an appropriate conformational propensity in the loop segment. Subtle variations in the covalent structure of the strand segments can prevent sheet formation. See, for example, Fisk, Powell, & Gellman (2000). The loop segment, however, need not be constructed from the same components as the strand segments. Several investigators have shown that non-peptide loops can allow anti-parallel β-sheet interactions between appended α-amino acid strands. Parallel β-sheet hairpins require a non-peptide loop because the strands must be linked C-terminus to C-terminus (or N-terminus to N-terminus). Anti-parallel sheet secondary structure has been documented in β-peptides containing both non-β-peptide and β-peptide linkers. See, for example, Chung et al. (2000).

BRIEF DESCRIPTION OF THE DRAWING FIGURES

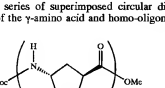
FIG. **1** is a model depicting the solid state crystal structure of compound 1. All hydrogen atoms, except those attached to nitrogen, have been omitted for clarity.

FIG. **2** is a model depicting the solid state crystal structure of compound 2. All hydrogen atoms, except those attached to nitrogen, have been omitted for clarity.

FIG. **3** is a graphic summary of selected NOEs for compound 2 (3.6 mM in CD_2Cl_2, 25° C.).

FIG. 4 is a graphic summary of selected NOEs for compound 3 (2 mM in pyridine-d5, 25° C).

FIG. 5 is a series of superimposed circular dichroism (CD) spectra of the γ-amino acid and homo-oligomers of



where n=1 to 6

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations and Definitions:

The following abbreviations and definitions are used throughout the specification and claims. Terms not expressly defined herein are to be given their conventional and accepted definition within the field of synthetic organic chemistry.

The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a fully saturated, straight, branched chain, or cyclic hydrocarbon radical, or combination thereof, and can include di- and multi-valent radicals, having the number of carbon atoms designated (e.g., C_1–C_{10} means from one to ten carbon atoms, inclusive). Examples of alkyl groups include, without limitation, methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)ethyl, cyclopropylmethyl, and homologs, and isomers thereof, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. The term "alkyl," unless otherwise noted, also includes those derivatives of alkyl defined in more detail below as "heteroalkyl" and "cycloalkyl."

The term "alkenyl" means an alkyl group as defined above containing one or more double bonds. Examples of alkenyl groups include vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), etc., and higher homologs and isomers.

The term "alkynyl" means an alkyl or alkenyl group as defined above containing one or more triple bonds. Examples of alkynyl groups include ethynyl, 1- and 3-propynyl, 3-butynyl, and the like, including higher homologs and isomers.

The terms "alkylene," "alkenylene," and "alkynylene," alone or as part of another substituent means a divalent radical derived from an alkyl, alkenyl, or alkynyl group, respectively, as exemplified by $—CH_2CH_2CH_2—$.

Typically, alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene groups will have from 1 to 24 carbon atoms. Those groups having 10 or fewer carbon atoms are preferred in the present invention. The term "lower" when applied to any of these groups, as in "lower alkyl" or "lower alkylene," designates a group having 10 or fewer carbon atoms.

"Substituted" refers to a chemical group as described herein that further includes one or more substituents, such as lower alkyl, aryl, acyl, halogen (e.g., alkylhalo such as CF_3), hydroxy, amino, alkoxy, alkylamino, acylamino, thioamido, acyloxy, aryloxy, aryloxyalkyl, mercapto, thia, aza, oxo, both saturated and unsaturated cyclic hydrocarbons, heterocycles and the like. These groups may be attached to any carbon or substituent of the alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene moieties. Additionally, these groups may be pendent from, or integral to, the carbon chain itself.

The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable, saturated or unsaturated, straight, branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and from one to three heteroatoms selected from the group consisting of O, N, Si, and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom(s) may optionally be quaternized. The heteroatom(s) O, N and S may be placed at any interior position of the heteroalkyl group. The heteroatom Si may be placed at any position of the heteroalkyl group, including the position at which the alkyl group is attached to the remainder of the molecule. Examples include $—CH_2—CH_2—O—CH_3$, $—CH_2—CH_2—NH—CH_3$, $—CH_2—CH_2—N(CH_3)—CH_3$, $—CH_2—S—CH_2—CH_3$, $—CH_2—CH_2—S(O)—CH_3$, $—CH_2—CH_2—S(O)_2—CH_3$, $—CH=CH—O—CH_3$, $—Si(CH_3)_3$, $—CH_2—CH=N—OCH_3$, and $—CH=CH—N(CH_3)—CH_3$. Up to two heteroatoms may be consecutive, such as in $—CH_2—NH—O—CH_3$ and $—CH_2—O—Si(CH_3)_3$. Explicitly included within the term "heteroalkyl" are those radicals that could also be described as "heteroalkylene" (i.e., a divalent radical, see next paragraph), and "heterocycloalkyl" (i.e., containing a cyclic group). The term "heteroalkyl" also explicitly includes unsaturated groups (i.e., heteroalkenyls and heteroalkynyls).

The term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified by $—CH_2—CH_2—S—CH_2CH_2—$ and $—CH_2—S—CH_2—CH_2—NH—CH_2—$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini. Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied.

The term "aryl" is used herein to refer to an aromatic substituent, which may be a single aromatic ring or multiple aromatic rings which are fused together, linked covalently, or linked to a common group such as a diazo, methylene or ethylene moiety. The common linking group may also be a carbonyl as in benzophenone. The aromatic ring(s) may include, for example phenyl, naphthyl, biphenyl, diphenylmethyl and benzophenone, among others. The term "aryl" encompasses "arylalkyl" and "substituted aryl." For phenyl groups, the aryl ring may be mono-, di-, tri-, tetra-, or penta-substituted. Larger rings may be unsubstituted or bear one or more substituents.

"Substituted aryl" refers to aryl as just described including one or more functional groups such as lower alkyl, acyl, halogen, alkylhalo (e.g., CF_3), hydroxy, amino, alkoxy, alkylamino, acylamino, acyloxy, phenoxy, mercapto, and both saturated and unsaturated cyclic hydrocarbons which are fused to the aromatic ring(s), linked covalently or linked to a common group such as a diazo, methylene, or ethylene moiety. The linking group may also be a carbonyl such as in cyclohexyl phenyl ketone. The term "substituted aryl" encompasses "substituted arylalkyl."

The term "acyl" is used to describe a ketone substituent, —C(O)R, where R is substituted or unsubstituted alkyl, alkenyl, alkynyl, or aryl as defined herein. The term "carbonyl" is used to describe an aldehyde substituent. The term "carboxy" refers to an ester substituent or carboxylic acid, i.e., —C(O)O— or —C(O)—OH.

The term "halogen" or "halo" is used herein to refer to fluorine, bromine, chlorine and iodine atoms.

The term "hydroxy" is used herein to refer to the group —OH.

The term "amino" is used to designate NRR', wherein R and R' are independently H, alkyl, alkenyl, alkynyl, aryl or substituted analogs thereof. "Amino" encompasses

"alkylamino," denoting secondary and tertiary amines, and "acylamino" describing the group RC(O)NR'.

The term "alkoxy" is used herein to refer to the —OR group, where R is alkyl, alkenyl, or alkynyl, or a substituted analog thereof. Suitable alkoxy radicals include, for example, methoxy, ethoxy, t-butoxy, etc. The term "alkoxyalkyl" refers to ether substituents, monovalent or divalent, e.g. —CH_2—O—CH_3 and —CH_2—O—CH_2—.

"ACHC"=3-aminocyclohexanecarboxylic acid

"ACPC"=3-aminocyclopentanecarboxylic acid

"BOC"=t-butoxycarbonyl

"BOP"=bis(2-oxo-3-oxazolidinyl) phosphonic acid

"Cbz"=carbobenzyloxycarbonyl

"CD"=far UV circular dichroism spectroscopy

"COSY"=correlated spectroscopy

"DCC"=N,N'-dicyclohexylcarbodiimide

"DCM"=dichloromethane

"DEAD"=diethyl azodicarboxylate

"DIC"=diisopropylcarbodiimide

"DIEA"=diisopropylethyl amine

"DMAP"=4-dimethylaminopyridine

"DMF"=dimethylformamide

"EDCI"=1-{3-(dimethylamino)propyl}-3-ethylcarbodiimide

"EDT"=ethanedithiol

"Fmoc"=9-fluorenylmethoxy carbonyl

"Fmoc-OSu"=9-fluorenylmethyl succinimidyl carbonate

"HOBt"=1-hydroxybenzotriazole

"HBTU"=2-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

"NMP"=N-methyl pyrrolidinone

"NMR"=nuclear magnetic resonance spectroscopy

"NOESY"=nuclear Overhauser effect spectroscopy

"PMA"=phosphomolybdic acid stain

"PyBOP"=benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

"ROESY"=rotational nuclear Overhauser effect spectroscopy"

"TFA"=trifluoroacetic acid

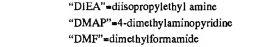
"THF"=tetrahydrofuran

"TLC"=thin-layer chromatography

"TOCSY"=total correlation spectroscopy

Overview of Compounds:

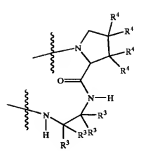
In a first embodiment, the invention is directed to an unnatural polypeptide compound containing at least one residue comprising a cyclically-constrained γ-amino acid residue. Here, the invention is directed to unnatural polypeptide compounds selected from the group consisting of:



(i)

wherein:

each Y is independently variable and is selected from the group consisting of a single bond or

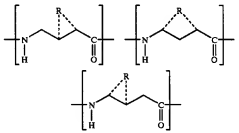


(ii)

where each R^3 is independently variable and is selected from the group consisting of hydrogen, linear or branched C_1–C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1–C_6-alkyl, and mono- or bicyclic heteroaryl-C_1–C_6-alkyl.

Each R^4 is selected from the same group of substituents as listed below for R^1.

Each X and each Z in formula (i) is independently variable and is selected from the group consisting of γ-amino acid residues, provided that at least one of X or Z is a cyclically-constrained γ-amino acid residue independently selected from the group consisting of:



(iii)

wherein R, together with the carbons to which it is attached, and further together with the β-position carbon in the γ-amino acid backbone where appropriate, independently defines a substituted or unsubstituted C_5 to C_{10} cycloalkyl, cycloalkenyl, or heterocycle moiety, the heterocycle moiety having 1, 2, or 3 heteroatoms selected from the group consisting of N, S, and O. Nitrogen and sulfur are the preferred heteratoms, nitrogen being the most preferred.

Each of "a," "c," and "d" is an independently variable positive integer. It is preferred that $a+c+d \geq 3$, and still more preferred that $a+c+d \geq 6$, although this is not required of the invention.

The "A" moiety of formula (i) is selected from the group consisting of hydrogen, hydroxy, an amino-terminus protecting group, and a carboxy-terminus protecting group. Thus, for amino termini, the "A" moiety is a hydrogen or an amino-terminus protecting group; for carboxy termini, the "A" moiety is a hydroxy group or carboxy-terminus protecting group. Salts of these compounds, pharmaceutical salts or otherwise, are included within the scope of the invention.

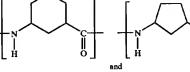
It is preferred that R in formula (iii), together with the carbons to which it is attached (and the carbon at the position β to the carbonyl group where appropriate), defines a substituted or unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

When R in formula (iii), together with the carbons to which it is attached (and together with the carbon at the position β to the carbonyl group where appropriate), defines a substituted cyclic moiety, the substituents on the cycloalkyl, cycloalkenyl, or heterocyclic moieties are independently selected from the group consisting of linear or branched C_1–C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1–C_6-alkyl, mono- or bicyclic heteroaryl-C_1–C_6-alkyl, $—(CH_2)_{n+1}—OR^2$, $—(CH_2)_{n+1}—SR^2$, $—(CH_2)_{n+1}—S(═O)—CH_2—R^2$, $—(CH_2)_{n+1}—S(═O)_2—CH_2—R^2$, $—(CH_2)_{n+1}—NR^2R^2$, $—(CH_2)_{n+2}—NHC(═O)R^2$, $—(CH_2)_{n+1}—NHS(═O)_2—CH_2—R^2$, $—(CH_2)_{n+1}—O—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—S—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—S(═O)—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—S(═O)_2—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—NH—(CH_2)_m—R^1$, $—(CH_2)_{n+2}—N—\{(CH_2)_m—R^1\}_2$, $—(CH_2)_{n+1}—NHC(═O)—(CH_2)_{m+1}—R^1$, $—(CH_2)_{n+1}—NHS(═O)_2—(CH_2)_m—R^1$; $—(CH_2)_n—OR$, $—(CH_2)_n—SR^2$, $—(CH_2)_n—S(═O)—CH_2—R^2$, $—(CH_2)_n—S(═O)_2—CH_2—R^2$, $—(CH_2)_n—NR^2R^2$, $—(CH_2)_n—NHC(═O)R^2$, $—(CH_2)_n—NHS(═O)_2—CH_2—R^2$, $—(CH_2)_n—O—(CH_2)_m—R^1$, $—(CH_2)_n—S—(CH_2)_m—R^1$, $—(CH_2)_n—S(═O)—(CH_2)_m—R^1$, $—(CH_2)_n—S(═O)_2—(CH_2)_m—R^1$, $—(CH_2)_n—NH—(CH_2)_m—R^1$, $—(CH_2)_n—N—\{(CH_2)_m—R^1\}_2$, $—(CH_2)_n—NHC(═O)—(CH_2)_m—R^1$, and $—(CH_2)_n—NHS(═O)_2—(CH_2)_m—R^1$; wherein R^2 is independently selected from the group consisting of hydrogen, C_3–C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteraryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1–C_6-alkyl, mono- or bicyclic heteroaryl-C_1–C_6-alkyl; and wherein R^1 is selected from the group consisting of hydroxy, C_1–C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1–C_6-alkylthio, C_1–C_6-alkylsulfinyl, C_1–C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1–C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1–C_6-alkylamino, carboxylic acid, carboxylate esters, carboxamide, mono- or di-C_1–C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1–C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the subsitutent(s) is selected from the group consisting of C_1–C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane, wherein m is an integer of from 2–6 and n is an integer of from 0–6.

It is generally preferred that one of X or Z is a γ-amino acid residue wherein R in formula (iii), together with the carbons to which it is attached (and the carbon at the position β to the carbonyl group where appropriate), independently defines a substituted or unsubstituted cyclopentyl, substituted or unsubstituted cyclohexyl, unsubstituted or N-substituted piperidinyl, or unsubstituted or N-substituted pyrrolidinyl.

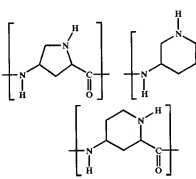
When R in formula (iii), together with the carbons to which it is attached and the carbon at the position β to the carbonyl group, defines an unsubstituted cyclic moiety, it is preferred that the moiety be selected from the group consisting of:

(iv)



and

(v)



As used in the specification and the claims, the word "independently," when referring to the nature of a variable substituent, explicitly means that each appearance of the defined substituent within a molecule can be different. Thus, for example, in a molecule according to the present invention such as $A\text{-}X_3\text{-}Z_3\text{-}B$ (where Y is a single bond, A is hydrogen, and B is hydroxy), each appearance of X and each appearance of Z can vary independently within the molecule. Thus, according to this explicit definition, the molecule $A\text{-}X_3\text{-}Z_3\text{-}B$ explicitly encompasses the molecule A-X'-X"-X'"-Z'-Z"-Z'"-B, where X' may the same as or different from X", and X'" may be the same as or different from X". Likewise, Z' may the same as or different from Z", and Z'" may be the same as or different from Z'".

As used herein, the terms "γ-amino acid" and "γ-amino acid residue" refer to any and all natural and unnatural γ-amino acids and their respective residues (i.e., the form of the amino acid when incorporated into a polypeptide molecule), without limitation. As used herein, the terms "amino-terminus protecting group" and "carboxy-terminus protecting group" refer to any chemical moiety capable of addition to and (optionally) removal from a reactive site (an amino group and a carboxy group, respectively, in this instance) to allow manipulation of a chemical entity at sites other than the reactive site. Protecting groups, and the manner in which they are introduced and removed are described, for example, in "Protective Groups in Organic Chemistry," Plenum Press, London, N.Y. 1973; and in "Methoden der organischen Chemie," Houben-Weyl, 4th edition, Vol. 15/I, Georg-Thieme-Verlag, Stuttgart 1974; and in Theodora W. Greene, "Protective Groups in Organic Synthesis," John Wiley & Sons, New York 1981. A characteristic of many protecting groups is that they can be removed readily, i.e., without the occurrence of undesired secondary reactions, for example by solvolysis, reduction, photolysis or alternatively under physiological conditions.

A host of protecting groups are known in the art. An illustrative, non-limiting list of protecting groups includes methyl, formyl, ethyl, acetyl, t-butyl, anisyl, benzyl,

trifluoroacetyl, N-hydroxysuccinimide, t-butoxycarbonyl, benzoyl, 4-methylbenzyl, thioanizyl, thiocresyl, benzyloxymethyl, 4-nitrophenyl, benzyloxycarbonyl, 2-nitrobenzoyl, 2-nitrophenylsulphenyl, 4-toluenesulphonyl, pentafluorophenyl, diphenylmethyl, 2-chlorobenzyloxycarbonyl, 2,4,5-trichlorophenyl, 2-bromobenzyloxycarbonyl, 9-fluorenylmethyloxycarbonyl, triphenylmethyl, and 2,2,5,7,8-pentamethylchroman-6-sulphonyl. The terms "amino-terminus protecting group" and "carboxy-terminus protecting group" as used herein are explicitly synonymous with such terms as "N-terminal capping group" and "C-terminal capping group," respectively. A host of suitable protecting and capping groups, in addition to those described above, are known in the art. For discussions of various different types of amino- and carboxy-protecting groups, see, for example, U.S. Pat. No. 5,221,736 (issued Jun. 22, 1993); U.S. Pat. No. 5,256,549 (issued Oct. 26, 1993); U.S. Pat. No. 5,049,656 (issued Sep. 17, 1991); and U.S. Pat. No. 5,521,184 (issued May 28, 1996).

Regarding salts of the subject compounds, compounds having at least one basic group or at least one basic radical, for example a free amino group, a pyrazinyl radical, or a pyridyl radical, may form acid addition salts. Thus, the invention encompasses acid addition salts of the subject compounds with (for example) inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid, or with suitable organic carboxylic or sulfonic acids, for example aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid or oxalic acid, or amino acids such as arginine or lysine, aromatic carboxylic acids, such as benzoic acid, 2-phenoxy-benzoic acid, 2-acetoxybenzoic acid, salicylic acid, 4-aminosalicylic acid, aromatic-aliphatic carboxylic acids, such as mandelic acid or cinnamic acid, heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid, aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxyethane-sulfonic acid, or aromatic sulfonic acids, for example benzene-, p-toluene- or naphthalene-2-sulfonic acid. When several basic groups are present mono- or poly-acid addition salts may be formed.

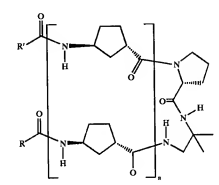
When the subject compounds have acidic groups, for example a free carboxy group, the invention encompasses metal and ammonium salts, such as alkali metal or alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines, such as tertiary monoamines, for example triethylamine or tri-(2-hydroxyethyl)-amine, or heterocyclic bases, for example N-ethylpiperidine or N,N'-dimethyl-piperazine.

Compounds of formula (i) having both acidic and basic groups can form internal salts. The salts may be pharmaceutically-acceptable salts or pharmaceutically-unacceptable salts.

Gamma Amino Acids and Polypeptides Formed Therefrom:

Molecular modeling studies performed by the inventors suggested that polypeptides containing γ-amino acid residues similar to trans-3-aminocyclopentanecarboxylic acid (trans-3-ACPC) residues would have a high propensity for γ-peptide parallel sheet secondary structure. Molecules exhibiting this type of secondary structure would be in stark contrast to the helical propensity previously documented for acyclic γ-amino acid residues.

Thus, molecules 1 and 2 were prepared. In each of these compounds, two (1S,3S)-trans-3-ACPC residues are linked via a D-prolyl-(1,1-dimethyl)-1,2-diaminoethyl unit:



1: n=1, R=R'=$OC(CH_3)_3$

2: n=1, R=R'=CH_2Ph

3: n=2, R=CH_2Ph, R'=$C(CH_3)_3$

The diamine linker portion of compounds 1, 2, and 3 has previously been shown to allow parallel β-sheet formation between attached α-amino acid residue strand segments. See Fisk, Powell, & Gellman (2000).

Crystal structures of 1 and 2 show that both molecules adopt the desired hairpin conformation in the solid state. See FIGS. 1 and 2, which are solid-state crystal structures of compounds 1 and 2, respectively. These results, particularly the similarity between two independent structures, show that the non-γ-peptide linker allows a parallel sheet hydrogen bonding pattern between attached γ-peptide strands.

Molecule 2 was examined by two-dimensional NMR methods in CD_2Cl_2 (3.6 and 5.7 mM, 25° C.) to evaluate the propensity for parallel γ-peptide sheet formation under dynamic conditions. Under these conditions, molecule 2 exhibits little or no aggregation in solution. (The amide proton shifts of 2 displayed minimal variation over the concentration range of from about 0.3 mM to about 10 mM in CD_2Cl_2, indicating that there is little or no self-association of 2 under these conditions.) Previous work with small oligoamides, including hairpin molecules that contain α- and/or β-amino acid residues has shown that intramolecular hydrogen bonding provides a modest drive for folding in nonpolar solvents, but that sheet-type hydrogen bonding will not occur unless both the strand and the turn segments have suitable conformational propensities.

A combination of COSY (correlated spectroscopy), TOCSY (total correlation spectroscopy), and ROESY (rotational nuclear Overhauser effect spectroscopy) spectra provided sufficient data to allow nearly all of the proton resonances from 2 to be unambiguously assigned. This, in turn, enabled the use of the amide proton chemical shift data to gain preliminary insight on folding. In nonpolar solvents C=O to H—N hydrogen bond formation causes an increase (up to 2–3 ppm) in the chemical shift of an amide proton (δNH). Equilibria between hydrogen bonded and non-hydrogen bonded states are usually rapid on the NMR time scale, and observed δNH values are therefore weighted averages of the contributing hydrogen bonded and non-hydrogen bonded states. For compound 2 in CD_2Cl_2, the

pattern of δNH values observed indicates that a significant population of the molecules adopt the conformation shown in FIG. 2; i.e., in solution, a significant proportion of compound 2 molecules adopt the same conformation as observed in the solid state. δNH-1 (5.52 ppm) and δNH (5.63 ppm) are consistent with little or no hydrogen bonding at these amide protons, while δNH-4 (7.04 ppm) and δNH-5 (7.24 ppm) indicate substantial hydrogen bond donation by these groups (using atom numbering as shown in FIG. 2).

More detailed structural insight was obtained from ROESY data for 2. Most informative among the short-range NOEs was one between the $C_\beta H$ of proline and the $C_\alpha H$ of the trans-3-ACPC residue attached to proline. This NOE showed that the tertiary amide linkage has the Z configuration in solution, as observed in both crystal structures shown in FIGS. 1 and 2. In addition, six NOEs between the two γ-amino acid residues (or immediately adjacent atoms) could be assigned unambiguously. See FIG. 3 for a graphic representation of these NOEs in compound 2. Five of these NOEs are consistent with the conformation observed for 2 in the solid state or modest distortions from this conformation: $C_\gamma H \rightarrow C_\alpha H$ (strong; 2.29 Å), $C_\gamma H \rightarrow$linker NH (weak; 3.64 Å), $C_\gamma H \rightarrow C_\gamma H$ (medium; 2.41 Å), $C_\gamma H \rightarrow$NH (weak; 3.72 Å), and phenacyl $CH_2 \rightarrow$NH (weak; 3.95 Å). (The distances given after NOE intensities were measured in the crystal structure of compound 2.)

The sixth nonadjacent NOE shown in FIG. 3, $C_\beta H \rightarrow$phenacyl CH_2 (weak), suggests that an alternative mode of interstrand interaction occurs to at least a small extent for 2 in CD_2Cl_2. This is because the shortest distance between protons on these two methylene groups is 5.91 Å in the crystal structure of 2.

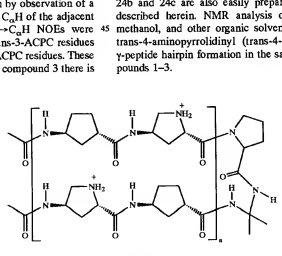
Molecule 3, which has two-residue γ-peptide strands on either side of the loop, was then synthesized and examined to determine whether parallel sheet secondary structure could propagate outward from the loop structure. Two-dimensional NMR analysis was carried out in pyridine-d_5 (2 mM, 25° C.) because compound 3 is nearly insoluble in CD_2Cl_2. Several key NOEs were unambiguously identified. See FIG. 4. The tertiary amide involving the proline nitrogen was shown to have the Z configuration by observation of a strong NOE between proline $C_\beta H$ and $C_\alpha H$ of the adjacent trans-3-ACPC residue. Strong $C_\gamma H \rightarrow C_\alpha H$ NOEs were observed between the inner pair of trans-3-ACPC residues and between the outer pair of trans-3-ACPC residues. These two NOEs indicate that in solutions of compound 3 there is a significant population of a hairpin conformation in which the parallel γ-peptide sheet involves all four trans-3-ACPC residues.

Thus, the present inventors have shown that the γ-peptides 1, 2, and 3 adopt sheet secondary structure in solution.

The utility of these compounds for probing protein interactions is great because, as noted above, the γ-peptides adopt structures analogous to those seen in natural proteins and peptides. Thus, the subject compounds mimic natural protein conformations in solution, but are resistant or immune to proteolytic degradation by proteases and peptidases. The cyclically-constrained γ-amino acid residues incorporated into homogeneous γ-peptide backbones are useful probes in the study of chemical and enzymatic interactions involving natural proteins. Also, the compounds disclosed herein add greatly to the γ-peptide field, in terms of both the number of alternative secondary structures that can be accessed and the intrinsic stability of those secondary structures. The subject compounds are useful probes because the cyclically-constrained residues create secondary structures with high conformational stability at short oligomer lengths that are also resistant to enzymatic degradation. The invention thus enhances the control over γ-peptide folding preferences, thereby providing a larger "toolbox" of probes to be used in investigating the function of naturally-occurring proteins.

Thus, the subject compounds are useful to implement a method of probing, disrupting, or mimicking binding interactions between two protein molecules or fragments thereof. The method comprises introducing to an in vivo, in vitro, or ex vivo reaction between two proteins, an unnatural polypeptide compound as described herein. Any effect of the added compound on thermodynamic or kinetic parameters of the binding interaction between the two protein molecules or fragments thereof is then measured. Because the subject compounds are conformationally similar to conventional α-polypeptides, but not subject to enzymatic degradation, the results provide valuable information regarding the interactions between large protein molecules.

Compounds 24a, an analog of 3, as well as higher analogs 24b and 24c are also easily prepared using the methods described herein. NMR analysis of these molecules in methanol, and other organic solvents, will show that the trans-4-aminopyrrolidinyl (trans-4-AP) residue supports γ-peptide hairpin formation in the same fashion as in compounds 1–3.



24a, n = 1
24b, n = 2
24c, n = 3

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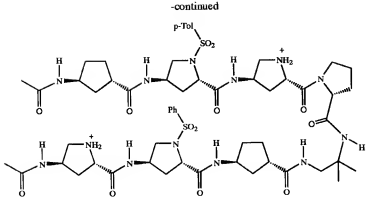
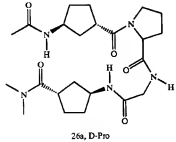


Because oligomers containing trans-4-AP display parallel sheet secondary structure in aqueous solution, diverse side-chains can be introduced into the rigidifying ring via sulfonylation of ring nitrogen atoms (e.g., as shown in compound 25). It has recently been shown that the ring nitrogen sulfonylation of pyrrolidine-based residues allows side-chain introduction in the β-peptide 12-helix, and this approach should function with equal success in the subject γ-peptides. See Lee et al. (2001). The design of 25 places hydrophobic side-chains across from one another, at the second residue of each strand. The crystallographic data for compounds 1 and 2 suggest that the parallel sheet conformation will be stabilized in water by clustering of the hydrophobic sulfonyl substituents.

Antiparallel γ-peptide secondary structure can be created by changing the linker used to connect strand segments. Initial studies will involve minimal hairpin molecules 26a–b, which have trans-3-ACHC residues in the strands.

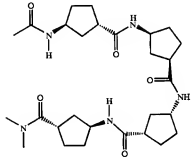


26a, D-Pro
26b, L-Pro

The prolyl-glycyl linker promotes strand interactions between α-amino acid residues. Gellman (1998)[6] and Ragothama et al. (1998). Molecular modeling indicates that this linker is suitable for γ-residues as well.

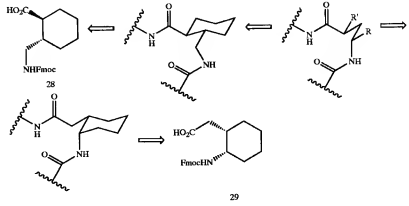
Molecular modeling also indicates that a heterochiral dimer of cis-3-ACPC will form the γ-peptide analog of the familiar β-turn seen in α-peptides. This hypothesis can be tested by examining tetra-γ-peptides like compound 27.

27



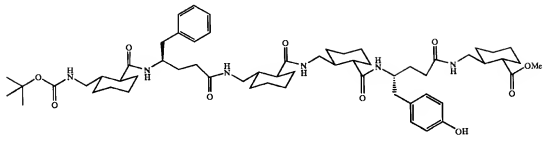
Functionally diverse antiparallel γ-peptide sheets that fold in water can thus be created by preparing analogs that contain amino-proline residues.

The only known type of γ-peptide helix was made exclusively with acyclic γ-amino acid residues; see Hintermann, Gademann, Jaun, Seebach (1998). Molecular modeling, however, indicates that two different cyclohexyl-rigidified residues, 28 and 29, will stabilize the γ-peptide 14-helix.



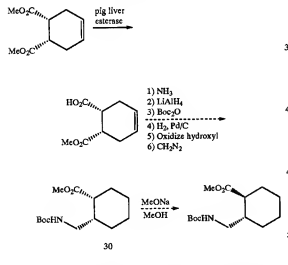
Both of these residues are available in enantiomerically pure form via straightforward extensions from or variations upon

31



the synthetic routes currently in use, see Appella et al. (1999)[a], to provide the β-amino acid trans-2-aminocyclohexanecarboxylic acid (trans-2-ACHC). For example, an N-protected form of cis-2-ACHC subjected to an Arndt-Eisterdt homologation reaction, Goodman et al. (1969), would then provide the N-protected version of 29. Preparation of the Boc-protected analog of 28 would start with the reported enzymatic desymmetrization of cis-4,5-cyclohexenedicarboxylic acid dimethyl ester. See Scheme 1 and Kobayashi, Kamiyama, & Ohno (1990)[b] and (1990)[b]:

SCHEME 1

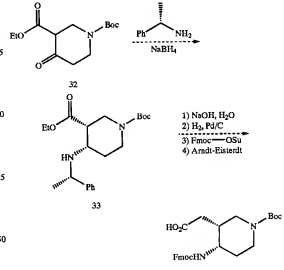


The half ester can be converted to either monoamide, and straightforward reactions will generate the protected cis-γ-amino acid 30. Kobayashi, Kamiyama, & Ohno (1990)[a] and (1990)[b] suggest that base-catalyzed epimerization will lead efficiently to the desired trans isomer, a protected form of 28.

Homo-oligomers of 28 and of 29, up to octamer length, are easily prepared using the linking chemistry described below. NMR analysis of these homooligomers, and co-oligomers of 28 and 29, in organic solvents, will reveal their conformational stability. Proton resonance overlap, however, may be too great to allow complete high-resolution structural analysis. If NMR analysis of these γ-peptides is fruitless, preparing γ-peptides containing a few scattered acyclic residues (e.g., hexamer 31) should enhance proton resonance dispersion.

The strategy of mixing cyclic and acyclic residues should also allow the fabrication of water-soluble γ-peptides that benefit from residue pre-organization. Various synthetic routes can then be used to make analogs that bear attachment sites for side-chains. For example, the route to trans-2-ACHC described above is applicable to commercially-available piperidine β-keto ester 32, which provides cis-amino ester 33 and ultimately protected γ-amino acid 34, an analog of 29 (Scheme 2):

SCHEME 2



Equally useful for purposes of the present invention are cyclic γ-amino acids that are not expected to promote 14-helix formation based on the conformational analysis above, e.g., γ-amino acids 35-40:

35

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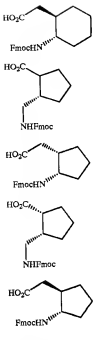


These studies provide unprecedented γ-peptide secondary structures (presumably helical). γ-Amino acids 35 and 36 are diastereomers of 28 and 29 that will disfavor the g^+g^+ torsion sequence along the (O=)CC_α—$C_\beta C_\gamma$ and $C_\alpha C_\beta$—$C_\gamma N$ backbone bonds, while γ-amino acids 35–40 contain cyclopentane rings. See Scheme 8 for a detailed synthesis of compound 40; see Scheme 9 for a detailed synthesis of the Boc-derivative of compound 36. The switch from trans-cyclohexyl to trans-cyclopentyl constraint among β-amino acids causes a profound change in β-peptide secondary structure preference (14- to 12-helix), presumably because the cyclopentyl constraint does not promote 60° angles about the backbone C_α—C_β bond. See Appella et al. (1996), (1997), (1999)[a,b], and Barchi et al. (2000). (See Scheme 10 for a detailed synthesis of the trans isomer of compound 34.) By extrapolation, residues derived from 37–40 are expected to disfavor the γ-peptide 14-helix. There is, however, a strong likelihood that these residues will give rise to other discrete secondary structure preference(s) because of the very limited residue conformational freedom.

Synthesis of the necessary γ-peptide building blocks is accomplished as follows. For example, 30 in Scheme 2 is a precursor to 35. Asymmetric opening of the anhydride of cis-1,2-cyclopentanedicarboxylic acid, Bolm, Schiffers, Dinter, & Gerlach (2000), will lead to 38 and 39. Arndt-Eisterdt homologation of Fmoc-protected trans-2-ACPC will generate Fmoc-protected 40. Pyrrolidine derivatives of 37–40 are available via analogous routes. See the various Schemes given in the Examples section.

Chemistry:

General. Melting points are uncorrected. CH_2Cl_2 was freshly distilled from CaH_2 under N_2. DMF was distilled under reduced pressure from ninhydrin and stored over 4 Å molecular sieves. Triethylamine was distilled from CaH_2 before use. Other solvents and reagents were used as obtained from commercial suppliers. For BOC removal, 4 M HCl in dioxane from was used. Column chromatography was carried out by using low air pressure (typically 6 psi) with 230–400 mesh silica gel 60. Routine ^{1}H-NMR spectra were obtained on a Bruker AC-300 and are referenced to residual protonated NMR solvent. Routine ^{13}C-NMR spectra were obtained on a Bruker AC-300 and are referenced to the NMR solvent. High resolution electron impact mass spectroscopy was performed on a Kratos MS-80RFA spectrometer with DS55/DS90.

Infrared Spectroscopy. Spectra were obtained on a Nicolet Model 740 FT-IR spectrometer. IR samples were prepared under anhydrous conditions; CH_2Cl_2 was freshly distilled from CaH_2, compounds and glassware were dried under vacuum for 1–2 days, and solutions were prepared under a nitrogen atmosphere. The pure solvent spectrum for a particular solution was subtracted from the sample spectrum prior to analysis. Peaks in the amide NH stretch region were baseline corrected, and analyzed without further manipulation.

NMR Spectroscopy:

Aggregation Studies. One-dimensional spectra for aggregation studies were obtained on a Bruker AC-300 spectrometer. Samples for aggregation studies were prepared by serial dilution from the most concentrated sample (50 mM or 27 mM). Dry compounds were dissolved in CD_2Cl_2 previously dried over 3 Å molecular sieves, and samples were prepared with dry glassware under a nitrogen atmosphere.

Conformational Analysis. NMR samples for conformational analysis were prepared by dissolving the dry compound in dry deuterated solvent under a nitrogen atmosphere. CD_2Cl_2 samples were then degassed by the freeze-pump-thaw method, and the NMR tubes were sealed under vacuum. Methanol samples were sealed with a close fitting cap and parafilm. COSY spectra were obtained on a Bruker AC-300 spectrometer. TOCSY, Braunschweiler et al. (1983); NOESY, Macura & Ernst (1980); and ROESY, Bothner-By et al. (1984) spectra were acquired on a Varian Unity-500 spectrometer using standard Varian pulse sequences and hypercomplex phase cycling (States-Haberkorn method), and the data were processed with Varian "VNMR" version 5.1 software. Proton signals were assigned via COSY and TOCSY spectra, and NOESY and ROESY spectra provided the data used in the conformational analyses. TOCSY spectra were recorded with 2048 points in t_1, 320 or 350 points in t_2, and 8 or 40 scans per t_2 increment. NOESY and ROESY spectra were recorded with a similar number of t_1 and t_2 points, and 32 and 40 scans per t_2 increment, depending on the sample concentration. The width of the spectral window examined was between 2000 and 4000 Hz. Sample concentrations for two-dimensional spectra were 2 mM in CD_2Cl_2 or 8 mM in CD_3OD or CD_3OH, or 2 mM in pyridine-d_5.

Far UV Circular Dichroism (CD). Data were obtained on a Jasco J-715 instrument at 20° C. In all CD plots contained herein, the mean residue ellipticity is presented on the vertical axis. Presenting the mean residue ellipticity is a standard practice in peptide chemistry wherein the intensity of each CD spectrum is normalized for the number of amide chromophores in the peptide backbone. Consequently, when the intensities of the maximum and minimum peaks characteristic of secondary structure formation increase with increasing chain length, this change represents an increase in the population of the secondary structure, rather than simply an increase in the number of chromophores present in each molecule.

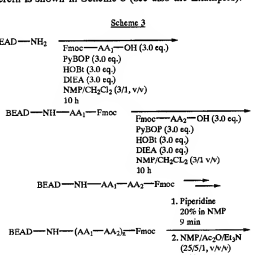
Solid-Phase and Solution-Phase Polypeptide Synthesis:

Construction of polypeptides using any type of γ-amino acid residue can be accomplished using conventional and widely recognized solid-phase or solution-phase synthesis.

Very briefly, in solid-phase synthesis, the desired C-terminal amino acid residue is linked to a polystyrene support as a benzyl ester. The amino group of each subsequent amino acid to be added to the N-terminus of the growing peptide chain is protected with Boc, Fmoc, or another suitable protecting group. Likewise, the carboxylic acid group of each subsequent amino acid to be added to the chain is activated with DCC, EDCI, PyBop, or another standard coupling reagent, and reacted so that the N-terminus of the growing chain always bears a removable protecting group. The process is repeated (with much rinsing of the beads between each step) until the desired polypeptide is completed. In the classic route, the N-terminus of the growing chain is protected with a Boc group, which is removed using trifluoracetic acid, leaving behind a protonated amino group. Triethylamine is used to remove the proton from the N-terminus of the chain, leaving a free amino group, which is then reacted with the activated carboxylic acid group from a new protected amino acid. When the desired chain length is reached, a strong acid, such as hydrogen bromide in trifluoracetic acid, is used to both cleave the C-terminus from the polystyrene support and to remove the N-terminus protecting group.

A representative solid-phase synthesis that can be used herein is shown in Scheme 3 (see also the Examples):

Scheme 3



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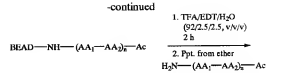


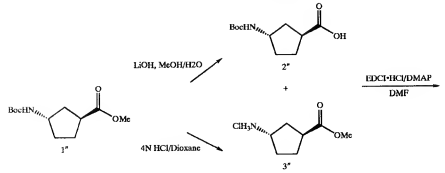
To begin each coupling, the Fmoc group on the resin bound amino acid/peptide is removed with 20% piperidine in N,N-dimethyl formamide (DMF). It is then rinsed and a protected amino acid is added which has been activated at its alpha carboxyl group. The activation is achieved by creating the N-hydroxybenzotriazole (HOBt) ester in situ. The activated A.A. and the resin bound A.A. are allowed to react in the presence of base to form a new peptide bond. This process is repeated until the desired peptide is assembled at the resin.

Once the peptide is complete, it is ready to be cleaved from the resin. This is accomplished using a mixture of trifluoroacetic acid (TFA) and any number of scavangers. Scavangers serve to neutralize cations which are formed during the removal of the side chain protecting groups. The cleavage solution is generally at least 82% TFA, and the rest a mixture of phenol, thioanisol, water, ethanedithiol (EDT), and triisopropylsilane (TIS). The peptide on the resin is allowed to react with the cleavage mixture for several hours, which then affords the peptide in solution. It can then be precipitated and washed in tert-butyl methyl ether, and analyzed or purified as desired.

Solid-phase peptide synthesis is, of course, widely employed and well known. There are several known variations on the above general approach. Consequently, solid-phase synthesis of peptides will not be described in any further detail. See, for example, "Peptide Synthesis, Structures, and Applications" © 1995 by Academic Press. Chapter 3 of this book addresses solid-phase peptide synthesis, and is incorporated herein by reference. Many of the subject γ-peptides can be made using the solid phase approaches described in this reference.

Solution phase synthesis, noted above, can also be used with equal success. For example, solution-phase synthesis of a γ-peptide chain can be accomplished as illustrated in the following coupling reaction:

Scheme 4



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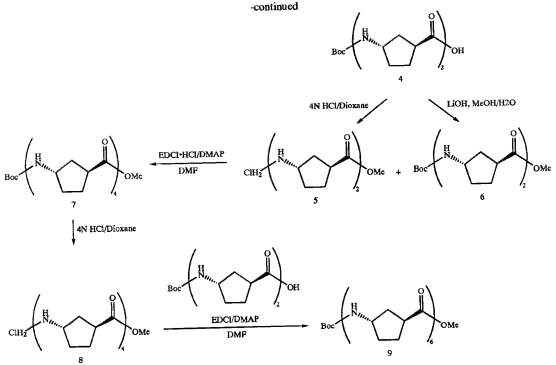


Compound 6: In 10 ml 5:1 MeOH:H_2O 4 (1.8 mmol) was dissolved. LiOH.H_2O (2.71 mmol) was added as a concentrated aqueous solution dropwise, maintaining the pH below 11. Solution stirred at RT 5 h. Neutralized with 0.5 M $NaHSO_4$ and evaporated MeOH. Acidified aqueous layer to pH 2. Extracted with EtOAc 2×. Dried organics with $MgSO_4$. 1.61 mmol of product was recovered, a white crystalline product. 93%.

Compound 7: To 0.56 mmol 4 was added 2 mL 4N HCl; in dioxane. After stirring at RT for one hour solvents were removed under a stream of nitrogen. Remaining residue was dissolved in DMF (3 mL). To the solution was added 6 (0.56 mmol), followed by DMAP (0.75 mmol), and EDCI (1.25 mmol). Solution stirred at RT 12 h. After about 1 h. a white precipate could be seen forming. 1 M HCl (2 mL) was added and the precipitate was filtered off and washed with water. Residue was then chromatographed with 20:1 CH_2Cl_2:MeOH. The product, a white powdery solid, was recovered in 88% yield. NMR was taken in 1:1 $CDCl_3$:CD_3OD due to poor solubility in other solvents.

All other compounds in Scheme 4 were synthesized in similar fashion. The hexamer 9 could not be chromatographed due to solubility issues, but was isolated pure by precipitation.

FIG. 5 is a series of superimposed CD spectra of the series leading from the monomer to the hexamer in the above coupling scheme. The spectra show the development of a characteristic minimum at about 190 nm, indicating the development of secondary conformation.

Adding Substituents to the Cyclic Moiety:

As noted above, the γ-peptides of the present invention can be substituted with any number of substituents, including hydroxy, linear or branched C_1–C_6-alkyl, alkenyl, alkynyl; hydroxy-C_1–C_6-alkyl, amino-C_1–C_6-alkyl, C_1–C_6-alkyloxy, C_1–C_6-alkyloxy-C_1–C_6-alkyl, amino, mono- or di-C_1–C_6-alkylamino, carboxamido, carboxamido-C_1–C_6-alkyl, sulfonamido, sulfonamido-C_1–C_6-alkyl, urea, cyano, fluoro, thio, C_1–C_6-alkylthio, mono- or bicyclic aryl, mono- or bicyclic heteraryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1–C_6-alkyl, heteroaryl-C_1–C_6-alkyl, and combinations thereof. Effecting such substitutions is well within the set of skills possessed by a synthetic peptide chemist.

For example, appending a sulfonamido moiety to the cylic backbone substituent can be accomplished in conventional fashion using Reaction 10. This reaction depicts the addition of a sulfonamido moiety to the cyclic back bone of a β-peptide. The same approach, however, will function using the analogous γ-peptide starting materials.

Compound 63: Compound 61 (90 mg) was dissolved in 4 N HCl in dioxane (2.0 ml). The reaction mixture was stirred for 1.5 hours. The dioxane was then removed in vacuo. The residue was dissolved in pyridine (2.0 ml), then cooled to 0° C. in an ice-bath.

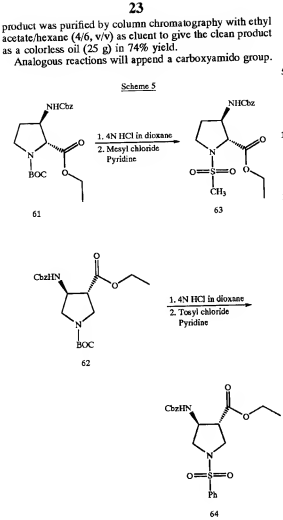
Methanesulfonylchloride (71 μl) was added dropwise. After the addition, the reaction mixture was stirred at room temperature for 12 hours. The pyridine was then removed in vacuo. The residue was taken up in ethyl acetate (50 ml). The mixture was washed with dilute brine (2×10 ml), dried over $MgSO_4$, and concentrated to give the clean product as a colorless oil (70 mg) in 82% yield.

Compound 64: Compound 62 (30 mg) was dissolved in 4 N HCl in dioxane (2.0 ml). The reaction mixture was stirred for 1.5 hours. The dioxane was then removed in vacuo. The residue was dissolved in pyridine (1.0 ml), then cooled to 0° C. in an ice-bath. Toluenesulfonylchloride (63 mg) was added in portions. After the addition, the reaction mixture was stirred at room temperature for 12 tours. The pyridine was then removed in vacuo. The residue was taken up in methylene chloride/diethyl ether (1/1, v/v, 100 ml). The mixture was washed with dilute brine (3×20 ml), dried over $MgSO_4$, and concentrated to give a liquid residue. The crude

product was purified by column chromatography with ethyl acetate/hexane (4/6, v/v) as eluent to give the clean product as a colorless oil (25 g) in 74% yield.

Analogous reactions will append a carboxyamido group.

Scheme 5



EXAMPLES

The following Examples are included solely to provide a more complete and consistent understanding of the invention disclosed and claimed herein. The Examples do not limit the scope of the invention claimed herein in any fashion.

See Schemes 6–10 for the structures of the various intermediate and final products.

General Procedures for All Examples: THF was distilled from sodium/benzophenone ketyl under N_2. Triethylamine was distilled from calcium hydride. All commercially available reagents and solvents were purchased from Aldrich (Milwaukee, Wis.) and used without further purification, except for 4 N HCl in dioxane, which was purchased from Pierce Chemical (Rockford, Ill.), and Fmoc-OSu, which was purchased from Advanced ChemTech (Louisville, Ky.). Analytical thin-layer chromatography (TLC) was carried out on Whatman TLC plates pre-coated with silica gel 60 (250 μm layer thickness). Visualization was accomplished using a UV lamp and phosphomolyhdic acid (PMA) stain (10% phosphomolyhdic acid in ethanol), or $KMnO_4$ stain. Column chromatography was performed on EM Science silica gel 60 (230–400 mesh). Solvent mixtures used for TLC and column chromatography are reported in v/v ratios.

{(1R,2S)-2-tert-Butoxycarboonylamino-cyclopentyl}-acetic acid (Boc-γ-ACPC-OH): (1S,2S)-2-Amino-cyclopentanecarboxylic acid (NH_2-ACPC-OH, JKM191, 5.18 g, 40.1 mmol) (See LePlae, Umezawa, Lee & Gellman (2001) *J. Org. Chem.* 66:5629–5632) was dissolved in methanol (409 mL). Triethylamine (11.16 mL, 80.2 mmol) was added via syringe followed by di-tert-butyl-dicarbonate (8.74 g, 40.1 mmol). The reaction mixture was stirred at room temperature for 2 h. The solvent was removed by rotary evaporation. The residue was diluted with ethyl acetate (250 mL), washed with 0.5 M $NaHSO_4$ (1×100), and saturated aqueous NaCl solution (1×100). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (7:3:0.3) hexane:ethyl acetate:acetic acid). Acetic acid was removed on the high-vacuum rotary evaporator. Benzene (2×100 mL) was added and removed on the rotary evaporator. The residue was dried under high vacuum overnight to give a white solid (Boc-ACPC-OH, JKM1103, 7.65 g, 83%). ^1H NMR (250 MHz, $CDCl_3$): δ=11.22 (br. s, 1H), 4.93 (br. s, 1H), 4.02 (br. s, 1H), 2.69 (br. s, 1H), 2.14–1.89 (m, 3H), 1.68 (quin., J=6.7 Hz, 2H), 1.51–1.35 (m, 10H) ppm.

(1R,2S)-2-tert-Butoxycarbonylamino-cyclopentanecarboxylic acid (Boc-ACPC-OH): JKM1103 (6.94 g, 30.3 mmol) was placed in an oven-dried flask with a clear-seal joint containing an oven-dried stir bar and dissolved in dry THF (61 mL) under a N_2 atmosphere. The solution was cooled to −14° C. with an ice/hrine bath. N-methylmorpholine (3.50 mL, 31.8 mmol) and isobutyl-chloroformate (4.13 mL, 31.8 mmol) were added via syringe. The mixture was stirred for 1 h and allowed to warm to 0° C. A white precipitate formed during this time. The flask was then fitted with an oven-dried "Diazald"-brand distillation apparatus (for generating diazomethane; see, for example, Hudlicky (1980) *J. Org. Chem.* 45:5377), and the joint was sealed with parafilm. A solution of KOH (12 g, 214 mmol) in H_2O (20 mL) and 2-methoxyethanol (16 mL) was placed in the well of the apparatus with a stir bar and heated to 75° C. A saturated solution of "Diazald"-brand reagent (N-methyl-N-nitroso-p-toluene-sulfonamide (19.5 g, 91 mmol) in diethyl ether (100 mL) was decanted into a dropping funnel attached to the apparatus. The vacuum adapter was fitted with a septum, and the system was placed under N_2. The cold finger was cooled to −78° C. with a dry ice/isopropanol mixture. The "Diazald" solution was then dropped into the KOH solution over a period of 30 min. The yellow diazomethane distilled over and condensed on the cold finger, and then dropped into the reaction mixture.

Once distillation of the diazomethane was complete, and the reaction mixture had a persistent yellow color, the "Diazald" apparatus was removed. The flask was stoppered, placed under N_2, and stirred for 4 h, being allowed to warm from 0° C. to room temperature. Acetic acid (1 mL) was then added to neutralize any excess diazomethane. The reaction mixture was diluted with diethyl ether (200 mL), washed with saturated aqueous $NaHCO_3$ (2x100 mL), aqueous HCl (1 N, 100 mL), and saturated aqueous NaCl solution (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (8:2 hexane:ethyl acetate) to give a pale yellow solid (Boc-ACPC-CHN_2, JKM1125, 4.75 g, 62%). ^1H NMR (250 MHz, $CDCl_3$): δ=5.58 (br. s, 1H), 4.70 (br. s, 1H), 3.99 (quin., J=6.3 Hz, 1H), 2.66 (br. s, 1H), 2.03–1.55 (m, 5H), 1.49–1.29 (m, 10H) ppm.

This solid (Boc-ACPC-CHN_2, JKMI125, 2.71 g, 10.68 mmol) was dissolved in water/dioxane (1/5, 480 mL), and the flask was covered in aluminum foil to exclude light. Silver benzoate (0.245 g, 10 mol %) was added as a catalyst, and the mixture was sonicated at room temperature under N_2 for 1 h. At 0° C., the solution was acidified to pH 2 with aqueous 0.5 M $NaHSO_4$. The solution was extracted with diethyl ether (4×150 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (6:4:0.3 hexane:ethyl acetate:acetic acid). Acetic acid was removed on the high-vacuum rotary evaporator. Benzene (2×200 mL) was added and removed on the rotary evaporator. The residue was dried under high vacuum overnight to give a yellowish white solid (Boc-γ-ACPC-OH, JKMI127, 2.36 g, 91%). This compound exists as slowly interconverting rotamers. ^1H NMR (300 MHz, $CDCl_3$): δ=11.63 (br. s, 1H), 6.53 (br. s, 1H), 5.04 (d, J=7.4 Hz, 1H), 3.59–3.46 (m, 1H), 2.65–2.55 (m, 1H), 2.28–2.23 (br. s, 1H), 2.07–1.93 (M, 3H), 1.66–1.24 (m, 12H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=178.04 (C), 157.84, 156.14 (C), 80.82, 80.75 (C), 58.20, 56.91 (CH), 42.84 (CH), 38.00 (CH_2), 32.38 (CH_2), 30.03 (CH_2), 28.45 ($3CH_3$), 21.51 (CH_2) ppm. MS-ESI: m/z=242.2 $\{M-H\}^-$, 485.3 $\{2M-H\}^-$.

Boc-(γ-$ACPC)_2$-OBn: This solid (Boc-γ-ACPC-OH, JKMI127, 1.00 g, 4.12 mmol) and $CsCO_3$ (1.34 g, 4.12 mmol) were dissolved in THF (41 mL). Benzyl bromide (0.53 mL, 4.44 mmol) was added, and the mixture was stirred at room temperature under N_2 covered in aluminum foil for 24 h. The mixture was diluted with ethyl acetate (200 mL) and washed with saturated aqueous $NaHCO_3$ (1×100 mL) and saturated aqueous NaCl (1×100 mL) solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (8:2 hexane:ethyl acetate) and dried overnight under high vacuum to give white solid (Boc-γ-ACPC-OBn, JKMI129, 0.58 g, 42%). ^1H NMR (300 MHz, $CDCl_3$): δ=7.40–7.30 (m, 5H), 5.12 (s, 2H), 4.59 (br. s, 1H), 3.57 (br. t, J=8.2 Hz, 1H), 2.65 (dd, J=15.8, 4.9 Hz, 1H), 2.32 (dd, J=15.6, 8.8 Hz, 1H), 2.11–1.88 (m, 3H), 1.70–1.59 (m, 2H), 1.43–1.21 (m, 11H) ppm.

The combined aqueous layers were acidified with acetic acid and extracted with ethyl acetate (3×75 mL) to isolate unreacted starting material (Boc-γ-ACPC-OH).

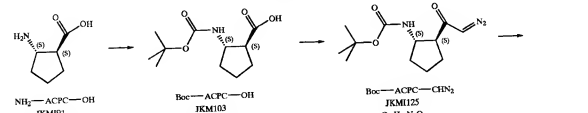
This white solid (Boc-γ-ACPC-OBn, JKMI129, 0.839 g, 2.52 mmol) was dissolved in 4 N HCl in dioxane (10 mL) and stirred under N_2 for three hours at room temperature. The solvent was blown off overnight under a stream of N_2. The residue was placed under high vacuum for one hour and then carried on without further purification (NH_2-γ-ACPC-OBn*HCl, JKMI133).

Boc-γ-ACPC-OH (JKMI127, 0.612 g, 2.52 mmol), NH_2-γ-ACPC-OBn*HCl (JKMI133, 0.679 g, 2.52 mmol), and 4-dimethylaminopyridine (1.076 g, 8.817 mmol) were dissolved in DMF (15 mL, anhydrous). The solution turned a pale orange color. To the solution was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.726 g, 3.78 mmol), and the reaction mixture was stirred under N_2 at room temperature overnight. The mixture was diluted with 100 mL ethyl acetate and washed with 0.5 M aqueous $NaHSO_4$ (1×75 mL), saturated aqueous $NaHCO_3$ (1×75 mL), and saturated aqueous NaCl (1×75 mL) solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (1:1 hexane:ethyl acetate) to give a white solid (Boc-(γ-$ACPC)_2$-OBn, JKMI135, 1.004 g, 87%). ^1H NMR (300 MHz, $CDCl_3$): δ=7.38–7.28 (m, 5H), 6.77 (d, J=7.0 Hz, 1H), 5.10 (s, 2H), 4.82 (d, J=7.8 Hz, 1H), 3.87 (quin., J=8.1 Hz, 1H), 3.56 (br. quin., J=7.8 Hz, 1H), 2.64 (dd, J=15.8, 4.9 Hz, 1H), 2.40–2.29 (m, 2H), 2.19–1.83 (m, 7H), 1.72–1.47 (m, 4H), 1.43–1.21 (m, 13H) ppm. MS-ESI: m/z=481.3 $\{M+Na\}^+$, 939.5 $\{2M+Na\}^+$.

Boc-(γ-$ACPC)_4$-OBn: Boc-(γ-$ACPC)_2$-OBn (JKMI135, 0.375 g, 0.819 mmol) was dissolved in methanol (8.2 mL), and the flask was flushed with N_2. To the flask was added Pd/C 10% (0.056 g), and the flask was attached to a Parr apparatus and shaken overnight at an H_2 pressure of 44 psi. The reaction mixture was filtered through a syringe filter and concentrated via rotary evaporation to give a white solid (Boc-(γ-$ACPC)_2$-OH, JKMI139, 0.289 g, 96%). The crude product was carried on without further purification.

Boc-(γ-$ACPC)_2$-OBn (JKMI135, 0.375 g, 0.819 mmol) was dissolved in 4N HCl in dioxane and stirred under N_2 at room temperature for 2 h. The solvent was blown off overnight under a stream of N_2. The residue was placed under high vacuum for 1 h. The residue (NH_2-(γ-$ACPC)_2$-OBn*HCl, JKMI141) was carried on without further purification.

Scheme 6



NH_2—ACPC—OH
JKMI91
$C_6H_{11}NO_2$
Mol. Wt.: 129.16

Boc—ACPC—OH
JKMI63
$C_{11}H_{19}NO_4$
Mol. Wt.: 229.27

Boc—ACPC—CHN_2
JKMI125
$C_{12}H_{19}N_3O_3$
Mol. Wt.: 253.30

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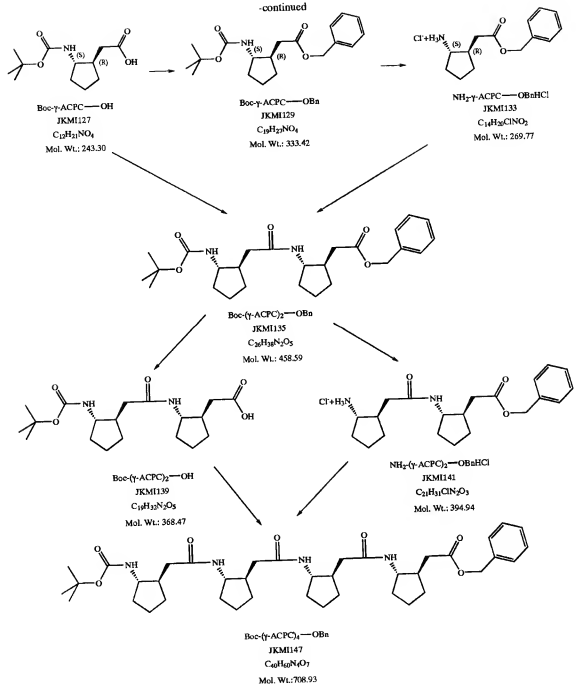


Boc-(γ-ACPC)$_2$-OH (JKMI139, 0.289 g, 0.786 mmol), NH_2-γ-ACPC-γ-ACPC-OBn•HCl (JKMI141), and 4-dimethylaminopyridine (0.350 g, 2.87 mmol) were dissolved in DMF (8.2 mL, anhydrous). To the solution was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.236 g, 1.23 mmol), and the reaction mixture was stirred under N_2 at room temperature overnight. A precipitate formed, and the solution turned pale orange during this time. Water (25 mL) was added to the reaction mixture to completely precipitate the product, which was then isolated by suction filtration. The solid was dissolved in CH_2Cl_2 (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3×25 mL). The combined organic extracts were washed with 0.5 M aqueous $NaHSO_4$ (1×25 mL), saturated aqueous $NaHCO_3$ (1×25 mL), and saturated aqueous NaCl (1×25 mL) solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The organic layers were pooled, washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated to give a white solid. The crude product was purified by column chromatography (CH_2Cl_2/

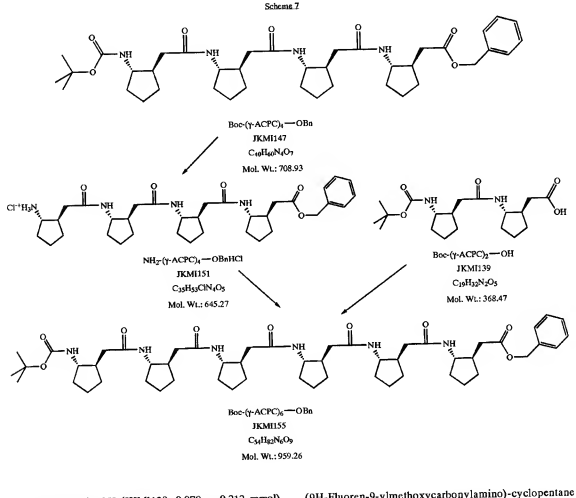
10% MeOH in CH_2Cl_2, loaded by adsorption to silica gel) to give a white solid (Boc-(γ-ACPC)$_4$-OBn, JKM1147, 0.306 g, 53%). ^{1}H NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD): δ=7.58 (br. s, 2H), 7.41–7.29 (m, 5H), 7.23 (br. s, 2H), 5.11 (s, 2H), 3.87–3.74 (m, 3H), 3.54–3.42 (m, 2H), 2.63 (dd, J=15.5, 4.3 Hz, 1H), 2.37–1.84 (m, 22H), 1.74–1.52 (m, 7H), 1.43–1.15 (m, 14H) ppm. MS-ESI: m/z=731.5 {M+Na}$^+$.

Boc-(γ-ACPC)$_6$-OBn: Boc-(γ-ACPC)$_4$-OBn (JKM1147, 0.150 g, 0.212 mmol) was dissolved in 4N HCl in dioxane (10 mL) and stirred under N_2 at room temperature for 2 h. The material fully dissolved, then a white precipitate formed. The solvent was blown off overnight under a stream of N_2. The residue was placed under high vacuum for 1 h. The residue (NH_2-(γ-ACPC)$_4$-OBn•HCl, JKM1151) was carried on without further purification.

acetate then dissolved in 5:1 $CHCl_3$:CF_3CH_2OH and washed with 0.5M $NaHSO_4$, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl solutions. A solid precipitated upon addition of the aqueous solutions. This solid was isolated by filtration and dried under vacuum to give a white solid (Boc-(γ-ACPC)$_6$-OBn, JKM1155, approximately 0.1 g). ^{1}H NMR (300 MHz, 5:1 CD_2Cl_2:CF_3CD_2OD): δ=7.37–7.34 (m, 5H), 6.95–6.82 (br. m, 4H), 6.65 (d, J=6.0 Hz, 1H), 5.15 (s, 1H), 5.10 (s, 2H), 3.93–3.89 (m, 2H), 3.88–3.58 (m, 3H), 3.59–3.40 (m, 11H), 2.57 (dd, J=15.6, 4.9 Hz, 1H), 2.36–2.26 (m, 3H), 2.13–1.83 (m, 15H), 1.70–1.63 (m, 8H), 1.41–1.18 (m, 26H) ppm. MALDI-TOF MS: m/z=981.5 {M+Na}$^+$.

{(1R,2S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-cyclopentyl}-acetic acid (Fmoc-γ-ACPC-OH): (1S,2S)-2-

Scheme 7



Boc-γ-(ACPC)$_2$-OH (JKM1139, 0.078 g, 0.212 mmol), NH_2-(γ-ACPC)$_4$-OBn•HCl (JKM1151), and 4-dimethylaminopyridine (0.091 g, 0.742 mmol) were dissolved in DMF (5 mL, anhydrous). To the solution was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.061 g, 0.318 mmol), and the reaction mixture was stirred under N_2 at room temperature for 48 h. The solvent was then removed under a stream of N_2 and then under high vacuum. The residue was diluted with H_2O. The insoluble material was isolated by filtration and dried under vacuum. This solid was washed with diethyl ether and ethyl

(9H-Fluoren-9-ylmethoxycarbonylamino)-cyclopentane carboxylic acid (See See LePlae, Umezawa, Lee & Gellman (2001) *J. Org. Chem.* 66:5629–5632) (Fmoc-ACPC-OH, JKM197, 0.56 g, 1.59 mmol) was placed in an oven-dried flask with a clear-seal joint containing an oven-dried stir bar and dissolved in dry THF (8 mL) under a N_2 atmosphere. The solution was cooled to −14° C. with an ice/brine bath. N-methylmorpholine (183 μL, 1.67 mmol) and isobutylchloroformate (216 μL, 1.67 mmol) were added via syringe. The mixture was stirred for 1 h and allowed to warm to 0° C. A white precipitate formed during this time. The flask was then

fitted with an oven-dried "Diazald"-brand apparatus, and the joint was sealed with parafilm. A solution of KOH (4 g, 71.4 mmol) in H_2O (6.7 mL) and 2-methoxyethanol (5.3 mL) was placed in the well of the apparatus with a stir bar and heated to 75° C. A saturated solution of "Diazald" reagent (1.02 g, 4.76 mmol) in diethyl ether (10 mL) was decanted into a dropping funnel attached to the apparatus. The vacuum adapter was fitted with a septum, and the system was placed under N_2. The cold finger was cooled to −78° C. with a dry ice/isopropanol mixture. The "Diazald" solution was then dropped into the KOH solution over a period of 30 min. The yellow diazomethane distilled over, condensed on the cold finger, and dropped into the reaction mixture. Once distillation of the diazomethane was complete, and the reaction mixture had a persistent yellow color, the "Diazald" apparatus was removed. The flask was stoppered, placed under N_2, and stirred for four h, being allowed to warm from 0° C. to room temperature. Acetic acid (1 mL) was then added to neutralize any excess diazomethane. An off-white precipitate formed at this time. The reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with saturated aqueous $NaHCO_3$ (2×100 mL), aqueous HCl (1N, 100 mL) and saturated aqueous NaCl solution (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (1% methanol in CH_2Cl_2, loaded by adsorption onto silica gel) to give a white solid (Fmoc-ACPC-CHN_2, JKMI113, 0.42 g, 71%). ^1H NMR (250 MHz, DMSO-d_6): δ=7.89 (d, J=7.0 Hz, 2H), 7.68 (d, J=7.3 Hz, 2H), 7.41 (t, J=7.4 Hz, 2H), 7.32 (t, J=7.4 Hz, 2H), 6.01 (s, 1H), 4.32–4.17 (m, 2H), 4.01–3.92 (m, 1H), 3.56–3.26 (m, 2H), 2.74–2.55 (m, 1H), 1.99–1.43 (m, 6H) ppm.

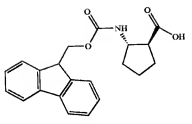
This solid (Fmoc-ACPC-CHN_2, JKMI113, 0.145 g, 0.387 mmol) was dissolved in water/dioxane (1/5, 20 mL), and the flask was covered in aluminum foil to exclude light. Silver benzoate (9 mg, 10 mol %) was added as a catalyst, and the mixture was sonicated at room temperature under N_2 for 1 h. At 0° C. the solution was acidified to pH 2 with aqueous HCl (1N). The solution was extracted with diethyl ether (4×25 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (1:1:0.3 hexane:ethyl acetate:acetic acid). Acetic acid was removed on the high-vacuum rotary evaporator. Benzene (2×100 mL) was added and removed on the rotary evaporator. The residue was recrystallized from CH_2Cl_2/hexane to obtain a white solid (Fmoc-γ-ACPC-OH, JKMI115, 0.041 g, 29%). ^1H NMR (300 MHz, CD_3OD): δ=7.78 (d, J=7.3 Hz, 2H), 7.64 (d, J=7.5 Hz, 2H), 7.38 (t, J=7.3 Hz, 2H), 7.30 (t, J=7.4 Hz, 2H), 4.41–4.31 (m, 2H), 4.21 (t, J=6.7 Hz, 1H), 3.54 (q, J=8.0 Hz, 1H), 2.51 (dd, J=14.6, 3.3 Hz, 1H), 2.19–1.93 (m, 5H), 1.72–1.25 (m, 5H) ppm.

(3S,4R)-3-Carboxymethyl-4-(9H-fluoren-9-ylmethoxycarbonylamino)-pyrrolidine-1-carboxylic acid tert-butyl ester (Fmoc-γ-APC(Boc)-OH): (4R,3S)-4-(9H-Fluoren-9-ylmethoxycarbonylamino)-pyrrolidine-1,3-dicarboxylic acid 1-tert-butyl ester (see Lee, LePlae, Porter & Gellman (2001) *J. Org. Chem.* 66:3597–3599) (Fmoc-APC(Boc)-OH, JKMI299, 5.05 g, 11.2 mmol) was placed in an oven-dried flask with a clear-seal joint containing an oven-dried stir bar and dissolved in dry THF (22 mL) under a N_2 atmosphere. The solution was cooled to −14° C. with an ice/brine bath. N-methylmorpholine (1.29 mL, 11.7 mmol) and isobutylchloroformate (1.52 mL, 11.7 mmol) were added via syringe. The mixture was stirred for 1 h and allowed to warm to 0° C. A white precipitate formed during this time. The flask was then fitted with an oven-dried "Diazald" apparatus, and the joint was sealed with parafilm. A solution of KOH (4.8 g, 85.7 mmol) in H_2O (8.0 mL) and 2-methoxyethanol (6.4 mL) was placed in the well of the apparatus with a stir bar and heated to 75° C. A saturated solution of "Diazald" reagent (7.2 g, 33.5 mmol) in diethyl ether (40 mL) was decanted into a dropping funnel attached to the apparatus. The vacuum adapter was fitted with a septum, and the system was placed under N_2. The cold finger was cooled to −78° C. with a dry ice/isopropanol mixture. The "Diazald" solution was then dropped into the KOH solution over a period of 30 min. The yellow diazomethane solution, condensed on the cold finger, and dropped into the reaction mixture. Once distillation of the diazomethane was complete, and the reaction mixture had a persistent yellow color, the "Diazald" apparatus was removed. The flask was stoppered, placed under N_2, and stirred for 4 h, being allowed to warm from 0° C. to room temperature. Acetic acid (1 mL) was then added to neutralize any excess diazomethane. The reaction mixture was diluted with diethyl ether (200 mL), washed with saturated aqueous $NaHCO_3$ (2×100 mL), aqueous HCl (1N, 100 mL), and saturated aqueous NaCl solution (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (1:1 hexane:ethyl acetate) to give a pale yellow solid (Fmoc-APC(Boc)-CHN_2, JKMI17, 4.44 g, 84%). This compound exists as slowly interconverting rotamers. ^1H NMR (300 MHz, $CDCl_3$): δ=7.82 (d, J=7.3 Hz, 2H), 7.59 (d, J=7.3 Hz, 2H), 7.42 (t, J=7.2 Hz, 2H), 7.33 (t, J=7.3 Hz, 2H), 5.60, 5.54 (br. s, 1H), 5.18, 5.12 (br. s, 1H), 4.80–4.47 (br. m, 3H), 4.22 (d, J=6.4 Hz, 2H), 3.72–2.99 (br. m, 5H), 1.71 (s, 1H), 1.47 (s, 9H) ppm.

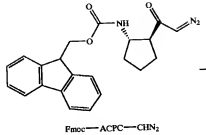
This solid (Fmoc-APC(Boc)-CHN_2, JKMI17, 4.44 g, 9.33 mmol) was dissolved in water/dioxane (1/5, 467 mL), and the flask was covered in aluminum foil to exclude light. Silver benzoate (0.214 g, 10 mol %) was added as a catalyst, and the mixture was sonicated at room temperature under N_2 for 1 h. At 0° C. the solution was acidified to pH 2 with aqueous 0.5M $NaHSO_4$. The solution was extracted with diethyl ether (4×200 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (1:1:0.3 hexane:ethyl acetate:acetic acid). Acetic acid was removed on the high vacuum rotary evaporator. Benzene (2×100 mL) was added and removed on the rotary evaporator. The residue was dried under high vacuum overnight to give a white solid (Fmoc-γ-APC(Boc)-OH, JKMI19, 2.61 g, 60%). This compound exists as slowly interconverting rotamers. ^1H NMR (300 MHz, CD_3OD): δ=7.77 (d, J=7.5 Hz, 2H), 7.63 (d, J=7.4 Hz, 2H), 7.37 (t, J=7.0 Hz, 2H), 7.30 (t, J=7.6 Hz, 2H), 4.42–4.36 (m, 2H), 4.16 (t, J=6.5 Hz, 1H), 3.86–3.80 (m, 1H), 3.74–3.63 (m, 2H), 3.06–3.00 (m, 2H), 2.53 (dd, J=15.7, 3.6 Hz, 1H), 2.42–2.22 (m, 2H), 1.45 (s, 9H) ppm.

Ac-(γ-APC-γ-ACPC)$_3$-NH_2: The γ-peptide Ac-(γ-APC-γ-ACPC)$_3$-NH_2 was synthesized from Fmoc-γ-APC(Boc)-OH (JKMI19) and Fmoc-γ-ACPC-OH (JKMI115) on a 25-μmol scale by standard methods on Rink amide AM resin (Applied Biosystems, Foster City, Calif.), using a Synergy automated synthesizer (Applied Biosystems). Amino acid (3 equiv.), HBTU (3 equiv.), and

Scheme 8

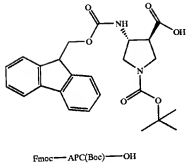


Fmoc—ACPC—OH
JKM#97
$C_{21}H_{21}NO_4$
Mol. Wt.:351.40

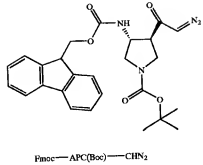


Fmoc—ACPC—CHN_2
JKMI113
$C_{22}H_{21}N_3O_3$
Mol. Wt.: 375.42

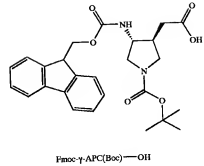
Fmoc-γ-ACPC—OH
JKMI115
$C_{23}H_{23}NO_4$
Mol. Wt.: 365.42



Fmoc—APC(Boc)—OH
JKMI299
$C_{25}H_{28}N_2O_6$
Mol. Wt.: 452.50

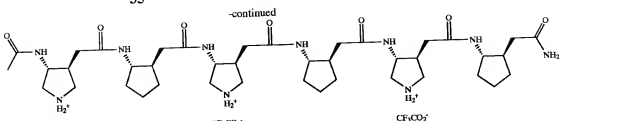


Fmoc—APC(Boc)—CHN_2
JKMII7
$C_{26}H_{28}N_4O_5$
Mol. Wt.: 476.52



Fmoc-γ-APC(Boc)—OH
JKMII9
$C_{27}H_{30}N_2O_6$
Mol. Wt.: 466.53

-continued

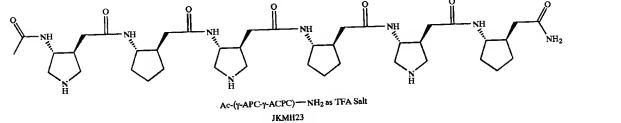


Ac-(γ-APC-γ-ACPC)—NH_2 as TFA Salt

JKMII23

$C_{19}H_{31}F_9N_{10}O_{13}$

Mol. Wt.: 1155.11



Ac-(γ-APC-γ-ACPC)—NH_2 as TFA Salt

JKMII23

$C_{41}H_{68}N_{10}O_7$

Mol. Wt.: 813.04

DIEA (6 equiv.) were used in each coupling cycle. Coupling cycles were 120 min. in duration, and piperdine deprotection cycles were approximately 60 min. in duration (actual deprotection time was regulated automatically by monitoring the conductivity trace). The resin-bound γ-peptide was cleaved and simultaneously deprotected by using 2 mL of 19:1 trifluoroacetic acid/H_2O and stirring for 4 h. The mixture was filtered through glass wool to remove the resin, and the filtrate was added to 7 mL of cold, anhydrous diethyl ether. The precipitate was collected by centrifugation. The γ-peptide was purified by reverse-phase HPLC on a C_{18}-silica semi-preparative column (5 μm; 10 mmx250 mm; Vydac, Hesperia, Calif.). The column was eluted with a gradient of acetonitrile in water (8–32%; 0.1% trifluoroacetic acid in each) at a flow rate of 5 mL/min. Collected fractions were pooled and lyophilized to produce a white powder (Ac-(γ-APC-γ-ACPC)$_3$-NH_2, JKMII23). ^1H NMR (500 MHz, CD_3OH): δ=8.54–8.51 (m, 2H), 8.46 (d, J=7.1 Hz, 1H), 8.25–8.19 (m, 3H), 7.79 (s, 1H), 6.95 (s, 1H), 4.21–4.16 (m, 2H), 3.83–3.78 (m, 3H), 3.70–3.60 (m, 6H), 3.16–3.04 (m, 6H), 2.57–2.51 (m, 6H), 2.41–2.31 (m, 6H), 2.17–1.89 (m, 16H), 1.74–1.63 (m, 6H), 1.54–1.47 (m, 3H), 1.45–1.36 (m, 3H) ppm. MALDI-TOF MS: calcd. for $C_{41}H_{68}N_{10}O_7$ {M} 812.5, found 813.4 $\{M+H\}^+$, 835.3 $\{M+Na\}^+$, 851.3 $\{M+K\}^+$.

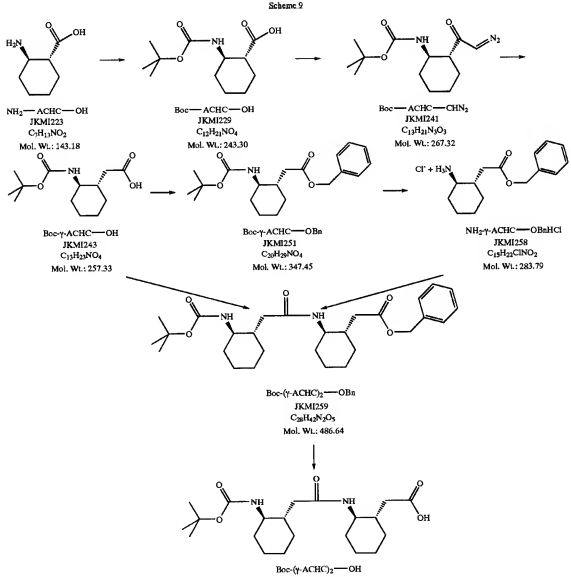
((1S,2R)-2-tert-Butoxycarbonylamino-cyclohexyl)-acetic acid (Boc-γ-ACHC-OH): (1R,2R)-2-Amino-cyclohexanecarboxylic acid (see Schinnerl, Murray, Langenhan, & Gellman (2003) *Eur J. Org. Chem.* 721–726) (NH_2-ACHC-OH, JKMI223, 2.18 g, 15.2 mmol) was dissolved in methanol (152 mL). Triethylamine (4.33 mL, 31.15 mmol) was added via syringe followed by di-tert-butyl-dicarbonate (3.41 g, 15.65 mmol). The reaction mixture was stirred at room temperature for 2 h. The solvent was removed by rotary evaporation. The residue was diluted with ethyl acetate (200 mL), washed with 0.5M $NaHSO_4$ (1×100 mL) and saturated aqueous NaCl solution (1×100). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (6:4:0.3) hexane:ethyl acetate:acetic acid). Acetic acid was removed on the high vacuum rotary evaporator. Benzene (2×100 mL) was added and removed on the rotary evaporator. The residue was dried under high vacuum overnight to give a white solid (Boc-ACHC-OH, JKMI229, 2.52 g, 68%). ^1H NMR (250 MHz, $CDCl_3$): δ=10.46 (br. s, 1H), 5.97 (br. s, 1H), 4.61 (br. s, 1H), 3.64 (br. s, 1H), 2.25 (td, J=11.3, 3.6, 2H), 2.08–1.95 (m, 4H), 1.75–1.16 (m, 11H) ppm.

(1S, 2S)-2-tert-Butoxycarbonylamino-cyclohexanecarboxylic acid (Boc-ACHC-OH, JKMI229, 3.61 g, 14.9 mmol) was placed in an oven-dried flask with a clear-seal joint containing an oven-dried stir bar and dissolved in dry THF (30 mL) under a N_2 atmosphere. The solution was cooled to −14° C. with an ice/brine bath. N-methylmorpholine (1.72 mL, 15.6 mmol) and isobutyl-chloroformate (2.02 mL, 15.6 mmol) were added via syringe. The mixture was stirred for 1 h and allowed to warm to 0° C. A white precipitate formed during this time. The flask was then fitted with an oven-dried "Diazald" apparatus, and the joint was sealed with parafilm. A solution of KOH (6 g, 107 mmol) in H_2O (10 mL) and 2-methoxyethanol (8 mL) was placed in the well of the apparatus with a stir bar and heated to 75° C. A saturated solution of "Diazald" reagent (9.56 g, 44.6 mmol) in diethyl ether (50 mL) was decanted into a dropping funnel attached to the apparatus. The vacuum adapter was fitted with a septum, and the system was placed under N_2. The cold finger was cooled to −78° C. with a dry ice/isopropanol mixture. The "Diazald" solution was then dropped into the KOH solution over a period of 30 min. The yellow diazomethane distilled over, condensed on the cold finger, and dropped into the reaction mixture. Once distillation of the diazomethane was complete, and the reaction mixture had a persistent yellow color, the "Diazald" apparatus was removed. The flask was

stoppered, placed under N_2, and stirred for 4 h, being allowed to warm from 0° C. to room temperature. Acetic acid (1 mL) was then added to neutralize any excess diazomethane. The reaction mixture was diluted with diethyl ether (200 mL), washed with saturated aqueous $NaHCO_3$ (2×100 mL), aqueous HCl (1N, 100 mL), and saturated aqueous NaCl solution (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (6:4 hexane:ethyl acetate) to give a pale yellow solid (Boc-ACHC-CHN_2, JKMI241, 1.24 g, 31%). ^1H NMR (300 MHz, $CDCl_3$): δ=5.45 (s, 1H), 4.66 (d, J=8.4 Hz, 1H), 3.54–3.50 (m, 1H), 2.35 (br. s, 1H), 2.04–1.73 (m, 5H), 1.51–1.17 (m, 12H) ppm.

This solid (Boc-ACHC-CHN_2, JKMI241, 1.24 g, 4.63 mmol) was dissolved in water/dioxane (1/5, 231 mL), and the flask was covered in aluminum foil to exclude light. Silver benzoate (0.106 g, 10 mol %) was added as a catalyst, and the mixture was sonicated at room temperature under N_2 for 1 h. At 0° C., the solution was acidified to pH 2 with aqueous 0.5M $NaHSO_4$. The solution was extracted with diethyl ether (4×150 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (6:4:0.3 hexane:ethyl acetate:acetic acid). Acetic acid was removed on the high-vacuum rotary evaporator. Benzene (2×150 mL) was added and removed on the rotary evaporator to give a white solid (Boc-γ-ACHC-OH, JKMI243, 1.273 g, wet). ^1H NMR (300 MHz, $CDCl_3$): δ=10.52 (br. s, 1H), 4.58 (d, J=9.7 Hz, 1H), 3.41–3.11 (m, 2H), 2.60 (dd, J 15.4, 5.5, Hz, 1H), 2.35–2.11 (m, 2H), 2.01–1.74 (m, 6H), 1.58–1.09 (m, 10H) ppm. MS-ESI: m/z=280.1 $\{M+Na\}^+$, 537.2 $\{2M+Na\}^+$, 256.1 $\{M-H\}^-$, 513.3 $\{2M-H\}^-$.

Scheme 9



((1R,2S)-2-tert-Butoxycarbonylamino-cyclohexyl)-acetic acid (Boc-γ-ACHC-OH, JKM1195) was prepared by starting with (1S,2S)-2-amino-cyclohexanecarboxylic acid (NH_2-ACHC-OH, JKM1181), see Schinnerl, Murray, Langenhan & Gellman (2001) *Eur. J. Org. Chem.* 721–726.

Boc-(γ-ACHC)$_2$-OH: This solid (Boc-γ-ACHC-OH, JKM1243, 0.30 g, 1.17 mmol) and $CsCO_3$ (0.40 g, 1.23 mmol) were dissolved in DMF (11.7 mL, anhydrous). Benzyl bromide (0.15 mL, 1.26 mmol) was added, and the mixture was stirred at room temperature under N_2 covered in aluminum foil for 24 h. The mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous $NaHCO_3$ (1×50 mL) and saturated aqueous NaCl (1×50 mL) solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (8.5:1.5 hexane:ethyl acetate) and dried overnight under high vacuum to give white solid (Boc-γ-ACHC-OBn, JKM1251, 0.34 g, 87%). ^1H NMR (300 MHz, $CDCl_3$): δ=7.40–7.27 (m, 5H), 5.12 (AB quar., ν_A=5.14, ν_B=5.09, J_{AB}=12.5 Hz, 2H), 4.49 (2, J=9.6 Hz, 1H), 3.27–3.17 (m, 1H), 2.64 (dd, J=15.8, 5.0 Hz, 1H), 2.21–1.95 (m, 3H), 1.83–1.63 (m, 4H), 1.48–1.10 (m, 12H) ppm.

This white solid (Boc-γ-ACHC-OBn, JKM1251, 0.202 g, 0.608 mmol) was dissolved in 4N HCl in dioxane (5 mL) and stirred under N_2 for three hours at room temperature. The solvent was blown off overnight under a stream of N_2. The residue was placed under high vacuum for one hour and then carried on without further purification (NH_2-γ-ACHC-OBn•HCl, JKM1258).

Boc-γ-ACHC-OH (JKM243, 0.156 g, 0.608 mmol), NH_2-γ-ACHC-OBn•HCl (JKM1258, 0.608 mmol), and 4-dimethylaminopyridine (0.222 g, 1.82 mmol) were dissolved in DMF (3.64 mL, anhydrous). To the solution was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.175 g, 0.912 mmol), and the reaction mixture was stirred under N_2 at room temperature overnight. The mixture was diluted with 50 mL ethyl acetate and washed with 0.5 M aqueous $NaHSO_4$ (1×50 mL), saturated aqueous $NaHCO_3$ (1×50 mL), and saturated aqueous NaCl (1×50 mL) solutions. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (1:1 hexane:ethyl acetate) to give a white solid (Boc-(γ-ACHC)$_2$-OBn, JKM1259, 0.224 g, 76%). ^1H NMR (300 MHz, $CDCl_3$): δ=7.36–7.27 (m, 5H), 5.94 (d, J=8.8 Hz, 1H), 5.11 (s, 2H), 4.63 (d, J=9.2 Hz, 1H), 3.60–3.50 (m, 1H), 3.20–3.10 (m, 1H), 2.54 (dd, J=16.3, 5.2, 1H), 2.30–2.14 (m, 2H), 2.03–1.59 (m, 12H), 1.48–0.95 (m, 16H).

Boc-(γ-ACHC)$_2$-OBn (JKM1259, 0.0875 g, 0.180 mmol) was dissolved in methanol (1.8 mL), and the flask was flushed with N_2. To the flask was added Pd/C 10% (0.013 g), and the flask was attached to a Parr apparatus and shaken overnight at an H_2 pressure of 44 psi. The reaction mixture was filtered through a syringe filter and concentrated on a rotary evaporator to give a white solid (Boc-(γ-ACHC)$_2$-OH, JKM1269). MS-ESI: m/z=397.2 $\{M+H\}^+$, 419.2 $\{M+Na\}^+$.

{(1R,2S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-cyclohexyl}-acetic acid (Fmoc-g-ACHC-OH): Boc-γ-ACHC-OH (JKM1195, 1.20 g, 4.95 mmol) was dissolved in 4N HCl in dioxane (10 mL) and stirred under N_2 for three hours at room temperature. The solvent was blown off overnight under a stream of N_2. The residue was placed under high vacuum for one hour and then carried on without further purification (NH_2-γ-ACHC-OH•HCl, JKM1113).

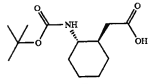
This residue (NH_2-γ-ACHC-OH•HCl, JKM1113, 4.95 mmol), was dissolved in acetone/H_2O (250 mL, 2:1) and cooled to 0° C., and Fmoc-OSu (1.67 g, 4.95 mmol) and $NaHCO_3$ (4.58 g, 54.5 mmol) were added. The turbid reaction mixture was stirred at 0° C. for 1 h and was then allowed to stir at room temperature for 12 h. The acetone was removed by rotary evaporation. The aqueous residue was diluted with H_2O (100 mL), and stirred at room temperature with diethyl ether (150 mL) for 1 h. The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (3×50 mL). The organic layer was discarded, and all the aqueous layers were combined, acidified with 1N aqueous HCl, and extracted with ethyl acetate (3×10 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated to obtain a white solid. The crude product was purified by column chromatography (1:1:0.3 hexanes:ethyl acetate:acetic acid) and recrystallized from $CHCl_3$/hexanes to give a white powder (Fmoc-γ-ACHC-OH, JKM1115, 0.924 g, 49%). ^1H NMR (300 MHz, CD_3OD): δ=7.77 (d, J=7.4 Hz, 2H), 7.58 (d, J=7.2 Hz, 2H), 7.37 (t, J=7.6 Hz, 2H), 7.28 (t, J=7.5 Hz, 2H), 4.57–4.15 (m, 3H), 3.70–3.64 (m, 1H), 3.18–3.11 (m, 1H), 2.48 (dd, J=15.4, 3.8, 1H), 2.39–2.30 (m, 1H), 1.73–1.15 (m, 9H) ppm.

(3S,4S)-3-Carboxymethyl-4-(9H-fluoren-9-ylmethoxycarbonylamino)-piperidine-1-carboxylic acid tert-butyl ester (Fmoc-γ-APiC(Boc)-OH): (3S,4S)-4-(9H-Fluoren-9-ylmethoxycarbonylamino)-piperidine-1,3-dicarboxylic acid 1-tert-butyl ester (LePlae, Umezawa, Lee & Gellman, supra) (Fmoc-APiC(Boc)-OH, JKM1129, 0.37 g, 0.79 mmol) was placed in an oven-dried flask with a clear-seal joint containing an oven-dried stir bar and dissolved in dry THF (4 mL) under a N_2 atmosphere. The solution was cooled to −14° C. with an ice/brine bath. N-methylmorpholine (92 μL, 0.83 mmol) and isobutylchloroformate (0.108 μL, 0.83 mmol) were added via syringe. The mixture was stirred for 1 h and allowed to warm to 0° C. A white precipitate formed during this time. The flask was then fitted with an oven-dried "Diazald"-brand apparatus, and the joint was sealed with parafilm. A solution of KOH (4 g, 71.4 mmol) in H_2O (6.7 mL) and 2-methoxyethanol (5.3 mL) was placed in the well of the apparatus with a stir bar and heated to 75° C. A saturated solution of "Diazald" reagent (0.51 g, 2.38 mmol) in diethyl ether (8 mL) was decanted into a dropping funnel attached to the apparatus. The vacuum adapter was fitted with a septum, and the system was placed under N_2. The cold finger was cooled to −78° C. with a dry ice/isopropanol mixture. The "Diazald" solution was then dropped into the KOH solution over a period of 30 min. The yellow diazomethane distilled over, condensed on the cold finger, and dropped into the reaction mixture.

Once distillation of the diazomethane was complete, and the reaction mixture had a persistent yellow color, the "Diazald" apparatus was removed. The flask was stoppered, placed under N_2, and stirred for 4 h, being allowed to warm from 0° C. to room temperature. Acetic acid (0.5 mL) was then added to neutralize any excess diazomethane. The reaction mixture was diluted with diethyl ether (100 mL), washed with saturated aqueous $NaHCO_3$ (2×50 mL), aqueous HCl (1N, 50 mL), and saturated aqueous NaCl solution (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (1:1 hexane:ethyl acetate) to give a pale yellow solid (Fmoc-APiC(Boc)-CHN_2, JKM1137, 0.154 g, 40%). ^1H NMR (300 MHz, $CDCl_3$): δ=7.73 (d, J=7.4 Hz, 2H), 7.56 (d, J=7.5 Hz, 2H), 7.37 (t, J=7.0 Hz, 2H), 7.28 (t, J=7.4 Hz, 2H), 5.48 (br. d, J=6.7 Hz, 1H), 4.36–3.93 (m, 7H), 3.20–2.60 (m, 2H), 2.10–1.90 (m, 3H), 1.46 (s, 9H) ppm.

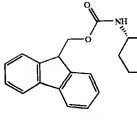
This solid (Fmoc-APiC(Boc)-CHN_2, JKMII37, 0.154 g, 0.314 mmol) was dissolved in water/dioxane (1/5, 18 mL), and the flask was covered in aluminum foil to exclude light. Silver benzoate (7.2 mg, 10 mol %) was added as a catalyst, and the mixture was sonicated at room temperature under N_2 for 1 h. At 0° C., the solution was acidified to pH 2 with aqueous 0.5M $NaHSO_4$. The solution was extracted with diethyl ether (4×25 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. The crude product was purified by column chromatography (7:3:0.3 hexane:ethyl acetate:acetic acid). Acetic acid was removed on the high-vacuum rotary evaporator. Benzene (2×100 mL) was added and removed on the rotary evaporator. The residue was dried under high vacuum overnight to give a white solid (Fmoc-g-APiC(Boc)-OH), JKMII47, 0.060 g, 40%). ^1H NMR (300 MHz, $CDCl_3$): δ=7.75 (d, J=6.5 Hz, 2H), 7.57 (d, J=6.2 Hz, 2H), 7.39 (t, J=7.4 Hz, 2H), 7.30 (t, J=7.0 Hz, 2H), 4.80–3.90 (m, 7H), 3.65 (br. s, 1H), 2.79 (br. s, 1H), 2.60–2.30 (br, m, 1H), 2.30–1.90 (br. m, 4H), 1.44 (s, 10H) ppm.

Scheme 10



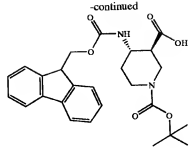
Boc-γ-ACHC—OH
JKMII95
$C_{13}H_{23}NO_4$
Mol. Wt.: 257.33

NH_2-γ-ACHC—OH
JKMII13
$C_8H_{15}NO_2$
Mol. Wt.: 157.21

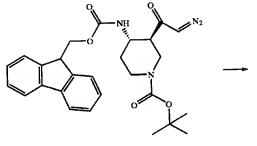


Fmoc-γ-ACHC—OH
JKMII15
$C_{23}H_{25}NO_4$
Mol. Wt.: 379.45

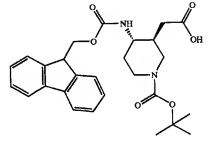
-continued



Fmoc—APiC(Boc)—OH
JKMII29
$C_{28}H_{30}N_2O_6$
Mol. Wt.: 466.53



Fmoc—APiC(Boc)—CHN_2
JKMII37
$C_{29}H_{30}N_4O_5$
Mol. Wt.: 490.55



Fmoc-γ-APiC(Boc)—OH
JKMII47
$C_{29}H_{32}N_2O_6$
Mol. Wt.: 480.55

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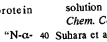
What is claimed is:

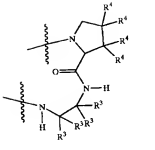
1. An unnatural polypeptide compound selected from the group consisting of:



(I)

wherein:

each Y is independently variable and is selected from the group consisting of a single bond or

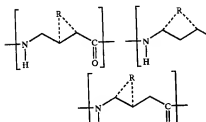


wherein each R^3 in Formula (ii) is independently variable and is selected from the group consisting of hydrogen, linear or branched C_1–C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1–C_6-alkyl, and mono- or bicyclic heteroaryl-C_1–C_6-alkyl;

each R^4 in Formula (ii) is selected from the group of substituents as listed hereinbelow for R^1;

each X and each Z in Formula (i) is independently variable and is selected from the group consisting of γ-amino acid residues, provided that at least one of X or Z is a substituted or unsubstituted cyclically-constrained γ-amino acid residue independently selected from the group consisting of:

(iii)



wherein R in Formula (iii), together with the carbons to which it is attached, independently defines a substituted or unsubstituted C_3 to C_{10} cycloalkyl, cycloalkenyl, or heterocycle moiety, the heterocycle moiety having 1, 2, or 3 heteroatoms selected from the group consisting of N, S, and O;

and further wherein substituents on the cyclically-constrained γ-amino acid residue of Formula (iii) are independently selected from the group consisting of linear or branched C_1–C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1–C_6-alkyl, mono- or bicyclic heteroaryl-C_1–C_6-alkyl, $—(CH_2)_{n+1}—OR^2$, $—(CH_2)_{n+1}—SR^2$, $—(CH_2)_{n+1}—S(=O)—CH_2—R^2$, $—(CH_2)_{n+1}—S(=O)_2—CH_2—R^2$, $—(CH_2)_{n+1}—NR^2R^2$, $—(CH_2)_{n+1}—NHC(=O)R^2$, $—(CH_2)_{n+1}—NHS(=O)_2—CH_2—R^2$, $—(CH_2)_{n+1}—O—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—S(=O)—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—S(=O)_2—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—S(=O)_2—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—NH—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—N—\{(CH_2)_m—R^1\}_2$, $—(CH_2)_{n+1}—NHC(=O)—(CH_2)_m—R^1$, $—(CH_2)_{n+1}—NHS(=O)_2—(CH_2)_m—R^1$; wherein R^2 is independently selected from the group consisting of hydrogen, C_1–C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1–C_6-alkyl, and mono- or bicyclic heteroaryl-C_1–C_6-alkyl; and wherein R^1 is selected from the group consisting of hydroxy, C_1–C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1–C_6-alkylthio, C_1–C_6-alkylsulfinyl, C_1–C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1–C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1–C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1–C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1–C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; and mono- di- or tri-substituted urea, wherein the substituent(s) is selected from the group consisting of C_1–C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane, and wherein m is an integer of from 2–6 and n is an integer of from 0–6;

wherein each "A" of Formula (i) is independently selected from the group consisting of hydrogen, hydroxy, an amino-terminus protecting group, and a carboxy-terminus protecting group;

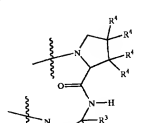
wherein each of "a," "c," and "d" is an independently variable positive integer; and

salts thereof.

2. The compound of claim 1, wherein a+c≧3.

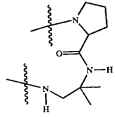
3. The compound of claim 1, wherein Y is a single bond and a+c≧3.

4. The compound of claim 1, wherein Y is:



and a+c≧3.

5. The compound of claim 1, wherein Y is:



and a+c≧3.

6. The compound of claim 1, wherein each R, together with the carbons to which it is attached, independently defines a substituted or unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

7. The compound of claim 1, wherein each R, together with the carbons to which it is attached, independently defines an unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

8. The compound of claim 1, wherein R, together with the carbons to which it is attached, defines a substituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom; and

substituents on the cycloalkyl, cycloalkenyl, or heterocycle moieties are independently selected from the group consisting of linear or branched C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl, $-(CH_2)_{n+1}-OR^2$, $-(CH_2)_{n+1}-SR^2$, $-(CH_2)_{m+1}-S(=O)-CH_2-R^2$, $-(CH_2)_{n+1}-S(=O)_2-CH_2-R^2$, $-(CH_2)_{n+1}-NR^2R^2$, $-(CH_2)_{n+1}-NHC(=O)R^2$, $-(CH_2)_{n+1}-NHS(=O)_2-CH_2-R^2$, $-(CH_2)_{n+1}-O-(CH_2)_m-R^1$, $-(CH_2)_{n+1}-S-(CH_2)_m-R^1$, $-(CH_2)_{m+1}-S(=O)-(CH_2)_m-R^1$, $-(CH_2)_{m+1}-S(=O)_2-(CH_2)_m-R^1$, $-(CH_2)_{n+1}-NH-(CH_2)_m-R^1$, $-(CH_2)_{m+1}-N-\{(CH_2)_m-R^1\}_2$, $-(CH_2)_{n+1}-NHC(=O)-(CH_2)_{n+1}-R^1$, $-(CH_2)_{n+1}-NHS(=O)_2-(CH_2)_m-R^1$, $-(CH_2)_n-OR$, $-(CH_2)_n-SR^2$, $-(CH_2)_m-S(=O)-CH_2-R^2$, $-(CH_2)_n-S(=O)_2-CH_2-R^2$, $-(CH_2)_n-NR^2R^2$, $-(CH_2)_n-NHC(=O)R^2$, $-(CH_2)_n-NHS(=O)_2-CH_2-R^2$, $-(CH_2)_m-O-(CH_2)_m-R^1$, $-(CH_2)_nS-(CH_2)_m-R^1$, $-(CH_2)_n-S(=O)-(CH_2)_m-R^1$, $-(CH_2)_n-S(=O)_2-(CH_2)_m-R^1$, $-(CH_2)_n-NH-(CH_2)_m-R^1$, $-(CH_2)_n-N-\{(CH_2)_m-R^1\}_2$, $-(CH_2)_n-NHC(=O)-(CH_2)_m-R^1$, and $-(CH_2)_n-NHS(=O)_2-(CH_2)_m-R^1$;

wherein R^2 is independently selected from the group consisting of hydrogen, C_1-C_6-alkyl, alkenyl, or alkynyl; mono- or bicyclic aryl, mono- or bicyclic heteraryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl-C_1-C_6-alkyl, mono- or bicyclic heteroaryl-C_1-C_6-alkyl; and

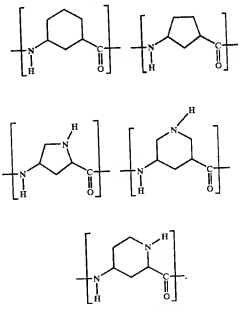
wherein R^1 is selected from the group consisting of hydroxy, C_1-C_6-alkyloxy, aryloxy, heteroaryloxy, thio, C_1-C_6-alkylthio, C_1-C_6-alkylsulfinyl, C_1-C_6-alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, amino, mono- or di-C_1-C_6-alkylamino, mono- or diarylamino, mono- or diheteroarylamino, N-alkyl-N-arylamino, N-alkyl-N-heteroarylamino, N-aryl-N-heteroarylamino, aryl-C_1-C_6-alkylamino, carboxylic acid, carboxamide, mono- or di-C_1-C_6-alkylcarboxamide, mono- or diarylcarboxamide, mono- or diheteroarylcarboxamide, N-alkyl-N-arylcarboxamide, N-alkyl-N-heteroarylcarboxamide, N-aryl-N-heteroarylcarboxamide, sulfonic acid, sulfonamide, mono- or di-C_1-C_6-alkylsulfonamide, mono- or diarylsulfonamide, mono- or diheteroarylsulfonamide, N-alkyl-N-arylsulfonamide, N-alkyl-N-heteroarylsulfonamide, N-aryl-N-heteroarylsulfonamide, urea; mono- di- or tri-substituted urea, wherein the substituent(s) is selected from the group consisting of C_1-C_6-alkyl, aryl, heteroaryl; O-alkylurethane, O-arylurethane, and O-heteroarylurethane;

m is an integer of from 2–6 and n is an integer of from 0–6; and

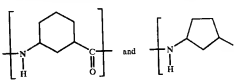
salts thereof.

9. The compound of claim 1, wherein each R, together with the carbons to which it is attached, independently defines a substituted or unsubstituted cyclopentyl, substituted or unsubstituted cyclohexyl, unsubstituted or N-substituted piperidinyl, or unsubstituted or N-substituted pyrrolidinyl.

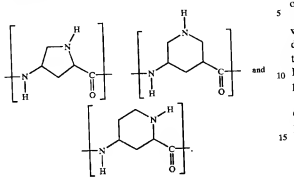
10. The compound of claim 1, wherein at least one of X or Z is a cyclically-constrained γ-amino acid residue independently selected from the group consisting of:



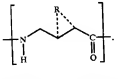
11. The compound of claim 1, wherein at least one of X or Z is a cyclically-constrained γ-amino acid residue independently selected from the group consisting of:



12. The compound of claim 1, wherein at least one of X or Z is a cyclically-constrained γ-amino acid residue selected from the group consisting of:



13. The compound of claim 1, wherein at least one of X or Z is a cyclically-constrained γ-amino acid residue selected from the group consisting of:

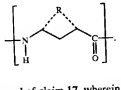


14. The compound of claim 13, wherein each R, together with the carbons to which it is attached, independently defines a substituted or unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

15. The compound of claim 13, wherein each R, together with the carbons to which it is attached, independently defines an unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

16. The compound of claim 13, wherein each R, together with the carbons to which it is attached, independently defines a substituted or unsubstituted cyclopentyl, substituted or unsubstituted cyclohexyl, unsubstituted or N-substituted piperidinyl, or unsubstituted or N-substituted pyrrolidinyl.

17. The compound of claim 1, wherein at least one of X or Z is a cyclically-constrained γ-amino acid residue independently selected from the group consisting of:

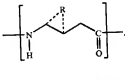


18. The compound of claim 17, wherein each R, together with the carbons to which it is attached, independently defines a substituted or unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

19. The compound of claim 17, wherein each R, together with the carbons to which it is attached, independently defines an unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

20. The compound of claim 17, wherein each R, together with the carbons to which it is attached, independently defines a substituted or unsubstituted cyclopentyl, substituted or unsubstituted cyclohexyl, unsubstituted or N-substituted piperidinyl, or unsubstituted or N-substituted pyrrolidinyl.

21. The compound of claim 1, wherein at least one of X or Z is a cyclically-constrained γ-amino acid residue independently selected from the group consisting of:



22. The compound of claim 21, wherein each R, together with the carbons to which it is attached, independently defines a substituted or unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

23. The compound of claim 21, wherein each R, together with the carbons to which it is attached, independently defines an unsubstituted C_5 to C_6 cycloalkyl, cycloalkenyl, or heterocycle moiety having a single nitrogen heteroatom.

24. The compound of claim 21, wherein each R, together with the carbons to which it is attached, independently defines a substituted or unsubstituted cyclopentyl, substituted or unsubstituted cyclohexyl, unsubstituted or N-substituted piperidinyl, or unsubstituted or N-substituted pyrrolidinyl.

25. The compound of claim 1, wherein when Y is a single bond, one of the "A" moieties is a hydrogen or a amino-terminus protecting group, and the other of the "A" moieties is a hydroxy or a carboxy-terminus protecting group; and when Y is not a single bond, both of the "A" moieties are hydrogens or amino-terminus protecting groups.

26. A method of probing, disrupting, or mimicking binding interactions between two protein molecules or fragments thereof, the method comprising:

in an in vivo, in vitro, or ex vivo reaction between the two proteins,

(a) introducing to the reaction an unnatural polypeptide compound according to claim 1; and then

(b) quantifying any effect of the added compound from step (a) on thermodynamic or kinetic parameters of the binding interaction between the two protein molecules or fragments thereof.

* * * * *

REFERENCE 7

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Unexpected Relationships between Structure and Function in α,β-Peptides: Antimicrobial Foldamers with Heterogeneous Backbones

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The development of synthetic foldamers, oligomers with discrete folding propensities, provides an excellent opportunity to explore relationships among covalent structure, molecular shape, and function.[1] Oligoamide foldamers with alternating α-amino acid residues and cyclic β-amino acid residues have recently been reported by Reiser et al. and by us to adopt helical conformations.[2,3] Here we describe an effort to generate antimicrobial α/β-peptides based on this folding behavior. Our findings suggest that common design assumptions in this field may exclude productive possibilities.

Natural host-defense peptides such as the magainins and cecropins are potent antibiotics that can adopt globally amphiphilic α-helical conformations, with lipophilic side chains segregated along one side of the helix and hydrophilic side chains along the other side.[4,5] These host-defense peptides are cationic, which causes attraction to the anionic surfaces of bacterial cells. Lipophilic peptide surfaces are thought to interact with hydrocarbon portions of lipids, thus disrupting the bilayer and compromising the bacterial membrane barrier. Many unnatural α-peptide sequences display antimicrobial activity,[4-6] as do designed β-peptides,[7] peptoids (*N*-alkyl glycine analogues),[8] and other molecules that have been designed to display globally amphiphilic conformations. [9-11]

Knowledge of helical residue periodicity is required to design an α/β-peptide sequence that will form a globally amphiphilic helix. Our previous work[3] indicated that β-residues with a five-membered ring constraint lead to short α/β-peptides that equilibrate between two internally H-bonded helices: the 11-helix (the numeral indicates the number of atoms in the H-bonded ring), with ca. 3 residues per turn, and the 14/15-helix, with ca. 4.5 residues per turn (Figure 1). We designed three sequence isomers, **1**−**3**, as potential antibiotics; similar lengths and cationic/lipophilic proportions have been successful among β-peptides.[7] For **1**, 11-helix formation would lead to discrete lipophilic and hydrophilic surfaces, but the 14/15-helix would not display global amphiphilicity (Figure 2). The situation is reversed in **2**: the 14/15-helix would be globally amphiphilic, but the 11-helix would not. In **3**, neither helical conformation would display global amphiphilicity.

Reversed-phase HPLC was used for initial assessment of designs **1**−**3**. Longer retention time is expected to correlate with a greater propensity to adopt a globally amphiphilic conformation. Such correlations have been observed among helical α-peptides,[12] and we have demonstrated similar behavior among helical β-peptides.[13] α/β-Peptides **1**−**3** were very well resolved by RP-HPLC.[14] As expected, the "scrambled" isomer **3** was least retained. The large difference between **1** and **2**, with **2** more strongly retained, suggests that the 14/15-helix is preferred relative to the 11-helix at this α/β-peptide length.

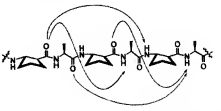

Figure 1. Hydrogen-bond patterns that define the helical secondary structures used to design potential antimicrobial peptides, with hydrogen bond from carbonyl groups to amide protons in the C-terminal direction. Blue arrows define the 11-helix; red arrows define the 14/15-helix.

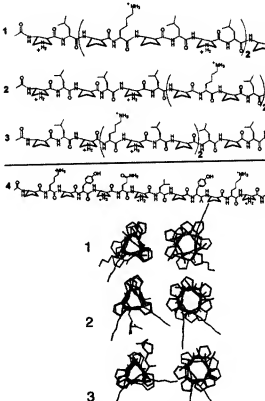

Figure 2. Axial views of predicted conformations (11-helix, left; 14/15-helix, right) for designed antimicrobial peptides **1**−**3** are shown. Residues colored red are positively charged.

The antimicrobial properties of **1**−**3** were assessed with four species (Table 1), including pathogens resistant to conventional antibiotics.[15] A modified host-defense peptide, Ala[8,13,18]-magainin-II amide, served as a positive control.[16] We were surprised to discover that **2**, designed to be globally amphiphilic in the 14/15-

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Table 1. Antimicrobial and Hemolytic Activities of Ala[8,13,18]-Magainin II Amide and α/β-Peptides (μg/mL)[13]

	E. coli	*B. subtilis*	*E. faecium*	*S. aureus*	max. concn without hemolysis
magainin	12.5	3.1	50	50	25
1	12.5	3.1	3.1−6.3	3.1	3.1
2	>100	6.3	25	50	1.6
3	6.3	6.3	6.3−12.5	12.5	50

helical conformation, is least active among the three α/β-peptide isomers. Both **1**, designed to be globally amphiphilic in the 11-helical conformation, and **3**, designed *not* to be globally amphiphilic in either helical conformation, are more active than **2** against the one Gram-negative species in our panel, *Escherichia coli*, and against the pathogenic *Enterococcus faecium* and *Staphylococcus aureus* strains.[17−19] The relatively high activity of **3** (comparable or superior to the magainin derivative against all four species) is particularly noteworthy in light of literature precedents on "scrambled" sequences among α- and β-peptides. Giangaspero et al. compared a 19-residue α-peptide designed to adopt a globally amphiphilic α-helix with a scrambled isomer; the latter displayed diminished activity relative to the former against a wide range of bacteria.[20] We have found even starker differences between β-peptides designed to form globally amphiphilic helices and their scrambled isomers, with the latter much less active.[7f,13]

Host-defense peptides are generally selective for killing prokaryotic cells relative to eukaryotic cells; in contrast, other peptides capable of forming amphiphilic α-helices, such as melittin, display indiscriminant toxicity.[21] Eukaryotic cell toxicity is often evaluated by monitoring human red blood cell lysis ("hemolysis"). We found that α/β-peptides **1** and **2** are at least as hemolytic as melittin, but scrambled isomer **3** is much less hemolytic and comparable to the magainin analogue in this regard (Table 1). This hemolysis trend, in contrast to the antimicrobial activity trend among **1**−**3**, parallels the effects of sequence scrambling on hemolytic behavior among helix-forming α- and β-peptides.[7f,13,20]

The unexpected finding that **2**, the sequence designed to be globally amphiphilic in the 14/15-helix conformation, is less toxic toward most bacteria than is the scrambled sequence, **3**, could indicate that the preference for 14/15-helical secondary structure inferred from RP-HPLC data (vide supra) is incorrect. NMR analysis of **1**−**3** was unsuccessful because of poor ^{1}H resonance dispersion, but nearly all backbone resonances of 15-mer **4** could be assigned in water and in methanol. Numerous $i,i+3$ NOEs were observed for **4**, many of which are consistent with either the 11-helical or the 14/15-helical conformation. However, molecular modeling suggests that α-residue C$_\alpha$H (i) → β-residue C$_\alpha$H (i+3) NOEs should be observed only for the 14/15-helix (i.e., not for the 11-helix).[3] Five of the seven possible NOEs of this type are observed for **4** in CD$_3$OH, a structure-promoting solvent, which suggests that the 14/15-helix is the preferred conformation. These NOEs are seen in water as well, but poorer resonance dispersion renders identification ambiguous in some cases.[22] Furthermore, α-residue C$_\alpha$H (i) → α-residue NH (i+2) NOEs are not expected for the 14/15-helix but should appear for the 11-helix (these NOEs are observed for shorter analogues of **1**−**4** (ref 3)); such $i,i+2$ NOEs are not observed for **4** in methanol or water, which argues against significant population of the 11-helix. The conclusion that **4** (and by extension **1**−**3**) favors the 14/15-helix relative to the 11-helix is supported by observation of a few $i,i+4$ NOEs that are predicted only for the 14/15-helix.[22] The contrast between these data and previous NMR studies of shorter α/β-peptides suggests that lengthening favors the 14/15-helix relative to the 11-helix.[3] This behavior supports the previously proposed analogy between helix formation in this class of α/β-peptides and in α-peptides,[3] because increasing length favors the α-helix (13-membered ring H-bonds) relative to the 3_{10}-helix (10-membered ring H-bonds).[23]

Among α/β-peptides **1**−**3** the most favorable behavior, high antimicrobial activity plus low hemolytic activity, is observed for scrambled isomer, **3**, and the least favorable behavior is observed for the isomer that is globally amphiphilic in the preferred 14/15-helical folding pattern, **2**. This result stands in contrast to the effects of sequence scrambling in α- or β-peptides.[7f,13,20] Antimicrobial oligomer design strategies have largely focused on the generation of globally amphiphilic conformations,[7−9] but our findings suggest that such approaches may unnecessarily limit the scope of the structures that are selected for evaluation.

Acknowledgment. This research was supported by the National Science Foundation (CHE-0240621). M.A.S. was supported in part by an NIH Molecular Biophysics Training Grant (GM08293-14). NMR spectrometers were purchased with partial support from NSF and NIH.

Supporting Information Available: NMR data and biological assay procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) HPLC conditions: C$_8$-silica analytical column; solvents: A: H$_2$O/0.1% TFA, B: 80% acetonitrile/20% H$_2$O/0.1% TFA; gradient: 1%B/min (20−60% B over 40 min); elution profile: **1**: 46.1% B; **2**: 55.4% B; **3**: 38.8% B.
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Pergamon

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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of (3*R*)-Carboxy Pyrrolidine (a β-Proline Analogue) and its Oligomer

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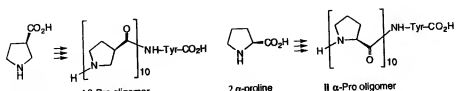
Abstract—A decamer of a β-amino acid analogue of L-proline, (3*R*)-carboxy pyrrolidine (β-proline), was synthesized from a readily available (*R*)-glycidol. It was found to possess a rigid secondary structure, as evidenced by its CD spectrum. The β-proline decamer, however, failed to bind to profilin, whereas the corresponding α-L-proline decamer bound tightly to this protein. © 2000 Published by Elsevier Science Ltd.

Since naturally occurring peptides are prone to being hydrolyzed by cellular proteases, their usefulness as therapeutic agents is very limited. Therefore, a new class of peptide analogues has been actively studied with the expectation that they would be resistant to proteolytic degradation and yet retain biological activity similar to that of their natural counterparts.[1] β-Amino acids possess an additional methylene unit between the amino and carboxyl groups, and their oligomers have been found to form an α-helical-like conformation, the L + 2 helix, as evidenced by NMR and X-ray crystallographic studies.[2–4] However, only a few of these designed oligomers have yet been shown to have biological activities similar to those of the naturally occurring peptides composed of α-amino acids.[5–7]

In the course of our ongoing research program of developing a new class of peptide analogues, we decided to simply replace the α-amino acid of a peptide with the corresponding β-amino acid and to examine its conformation and biological activity, and we chose a proline oligomer as our target peptide.

Yuki and co-workers have previously prepared polymers of β-proline analogue (3-carboxy pyrrolidine) and indicated that these polymers exist in random conformations.[8] Seebach et al. have prepared oligomers of β^2- and β^3-homoprolines (2-carboxymethyl- and 2-carboxyethyl-pyrrolidine, respectively) and suggested that they form rigid secondary structures.[9] Recently, Gellman et al. have reported that oligomers of a β-proline analogue, (3*S*)-carboxy pyrrolidine, form a rigid secondary structure, as indicated by their CD spectra.[10] The report from Gellman's group has prompted us to report our preliminary work on the synthesis and biological evaluation of an oligomer of a different β-proline analogue, (3*R*)-carboxy pyrrolidine **I**, which is an enantiomer of Gellman's compound. In this communication, we will describe a facile synthesis of **I** and the biological evaluation of its decamer **I** as a potential ligand of profilin.

Profilin interacts with a variety of biological molecules, including actin monomers and poly-proline rich sequences of proteins, and it plays a key role in regulating the



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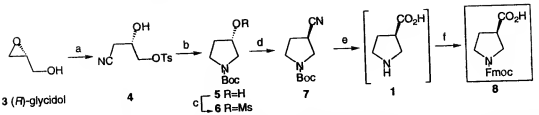

Scheme 1. Reagents and conditions: (a) ref 18; (b) (1) H_2/Raney® Ni/EtOH; (2) Boc_2O/Et_3N/MeOH (70% in two steps); (c) $MsCl/Et_3N/CH_2Cl_2$ (93%); (d) LiCN/DMF (84%); (e) concd HCl; (f) FmocCl/Na_2CO_3/H_2O–dioxane (72% in two steps).

dynamics of actin polymerization, which is responsible for many motile process in live cells.[11] Although the physiological significance of profilin binding to poly-proline is still unknown, the binding site on profilin was found to lie in a shallow groove between the N- and C-terminal helices and the underlying β-sheet.[12] In water, poly-proline forms a left-handed helix with a *trans*-peptide bond and three residues per turn (a type II helix), whereas in organic solvents it is known to form a right-handed *cis* helix (type I).[13] A recent X-ray crystallographic study of profilin complexed with an L-α-proline decamer has shown that the binding is due to specific hydrogen bonding between the amido carbonyl group of the proline oligomer and the NH group of profilin as well as the hydrophobic interaction between the pyrrolidine framework of α-L-prolines and the tryptophane residues of profilin.[14] We therefore planned to synthesize decamers of a β-proline analogue, (3*R*)-carboxyl pyrrolidine, as a β-peptide and of α-proline as a control and to evaluate the binding affinities of these decamers for a recombinant amoeba profilin in order to examine the possibility that β-peptide can substitute for the natural peptide.

Synthesis of β-Proline: (3*R*)-Carboxy Pyrrolidine

Although several synthetic procedures have been reported for the preparation of the optically active β-proline analogue 3-carboxylic pyrrolidine: by optical resolution of the racemic compounds,[8] from *trans* (4*R*)-hydroxy-L-proline[15] as used by Gellman et al.,[9] from L-aspartic acid[16] or by microbial optical resolution of a cyclobutenone derivative,[17] we decided to develop a new and facile synthetic route because we were interested in oligomers of a β-proline analogue with (3*R*) stereochemistry (see **1** and **2**).

Our synthesis of **1** started with a commercially available (*R*)-glycidol **3** (Scheme 1). According to the Sharpless procedure,[18] **3** was converted to the 3-nitrile derivative **4**, which was then reductively cyclized[19] to form a pyrrolidine derivative **5**.[20] The 3-OH group of **5** was mesylated and subsequently displaced with a nitrile group to give **7**.[20] The nitrile group of **7** was hydrolyzed with concentrated HCl to give **1**, which was isolated as the N-Fmoc derivative **8**.[20] Oligomers of **1** and **2** were prepared by standard solid-phase Fmoc chemistry,[21] and the L-tyrosine residue was incorporated into the C-terminus of each peptide for future tracer experiments.

Binding Study of Profilin and the Decamers I and II

Because the binding of poly(α-L-proline) enhances the intrinsic tryptophan fluorescence of profilin, we used fluorescence spectroscopy to evaluate the affinity of the decamers **I** and **II** for the purified *Acanthamoeba* profilin-I.[22] As expected, the decamer of α-proline **II** showed an increased fluorescence (excitation wavelength = 295 nm; emission wavelength = 300–380 nm) that is consistent with previous observations of the L-proline decamer. However, no significant increase in fluorescence was observed when the decamer of β-proline **I** was mixed

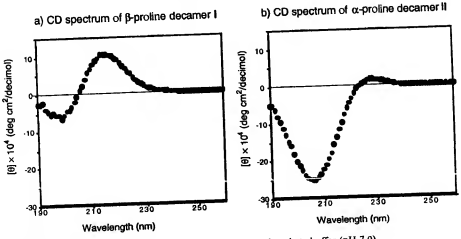

Figure 1. CD spectral data of decamers I and II (0.5 mM) in 10 mM potassium phosphate buffer (pH 7.0).

with profilin at concentrations as high as 100 mM, whereas the maximum fluorescence of profilin was achieved in the presence of α-proline decamer **II** at 318 nM.

CD Spectra of Decamers I and II

The decamer of α-L-proline **II** showed a CD spectrum typical of a type II poly (L-proline) helix, with a small positive band at 228 nm and a large negative band at 208 nm, in 10 mM potassium phosphate buffer (pH 7.0); these values are in good agreement with the reported[22] figures (Fig. 1b). In contrast, the other decamer of β-proline **I** had a large positive band at 215 nm and a negative band at 198 nm in the same buffer solution (Fig. 1a), yielding a curve opposite to that reported by Gellman et al. for the enantiomer of **I**,[10] indicating that the β-proline decamer **I** may have a rigid, ordered conformation.[23]

Summary

We have developed an efficient synthetic route for a β-proline analogue, (3*R*)-carboxy pyrrolidine, from an optically active (*R*)-glycidol employing a CN as a facile source of aminomethyl and carboxylate groups. The resulting decameric peptide indicated a rigid secondary structure based on its CD spectrum; however, it failed to bind to profilin, which shows a tight hydrogen bonding to the amido backbone of the α-proline decamer **II**.

Acknowledgements

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20. Compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 9H), 1.80 (br, 2H), 3.15–3.38 (m, 4H), 4.21 (br d, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 33.1, 33.6, 43.3, 53.7, 53.9, 69.4, 70.2, 79.1, 154.6; HRMS FAB calcd for $C_9H_{19}NO_5$ $(M+H)^+$ 188.1287, found 188.1288. Coumpound **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.05 (br, 2H), 2.94 (s, 3H), 3.32–3.54 (m, 4H), 5.13 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.3, 31.5, 32.4, 38.4, 43.1, 43. 5, 51.6, 52.0, 79.5, 80.1, 154.5; HRMS FAB calcd for $C_{10}H_{20}NO_7S$ $(M+H)^+$ 266.1062, found 266.1060. Compound **7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 2.06–2.26 (m, 2H), 3.06 (dt, J = 6.6, 13.5 Hz), 3.32–3.64 (m, 4H), 5.13 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.4, 28.2, 29.1, 29.9, 44.2, 48.6, 79.9, 119.8, 153.6; HRMS FAB calcd for $C_{10}H_{17}N_2O_2$ $(M+H)^+$ 197.1290, found 197.1289. Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.23 (m, 2H), 3.15 (dt, J = 7.2, 14.1 Hz), 3.48–3.58 (m, 2H), 3.72 (d, 2H, J = 6.9 Hz), 4.23–4.27 (m, 1H), 4.33–4.43 (m, 2H), 7.31 (dd, 2H, J = 7.2, 9.0 Hz), 7.40 (dd, 2H, J = 7.5 Hz), 7.60 (d, 2H, J = 7.5 Hz), 7.76 (d, 2H, J = 7.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 47.4, 61.3, 65.95, 65.99, 67.5, 99.0, 119.9, 125.0, 127.0, 127.6, 141.3, 144.0, 154.9, 177.3; HRMS FAB calcd for $C_{20}H_{20}NO_4$ $(M+H)^+$ 338.1392, found 338.1392.
21. Both peptides **I** and **II** were prepared by the Core Facility at Johns Hopkins University School of Medicine using a standard Fmoc chemistry on a solid phase and were HPLC-purified (>98%).
22. The buffer solution for intrinsic fluorescence assay contained: 5 μM profilin-1, 75 mM KCl, 10 mM Tris, pH 7.5, 1 mM NaN_3; for the detailed experimental procedure, see Petrella, E. C.; Machesky, L. M.; Kaiser, D. A.; Pollard, T. D. *Biochemistry* **1996**, *35*, 16535.
23. Further study of the conformation by NMR is in progress in order to determine the conformational similarity or difference between the oligomers of β-proline **I** and the corresponding α-proline counterpart **II**

RELATED PROCEEDINGS APPENDIX

There are no related appeals, interferences, or other proceedings.